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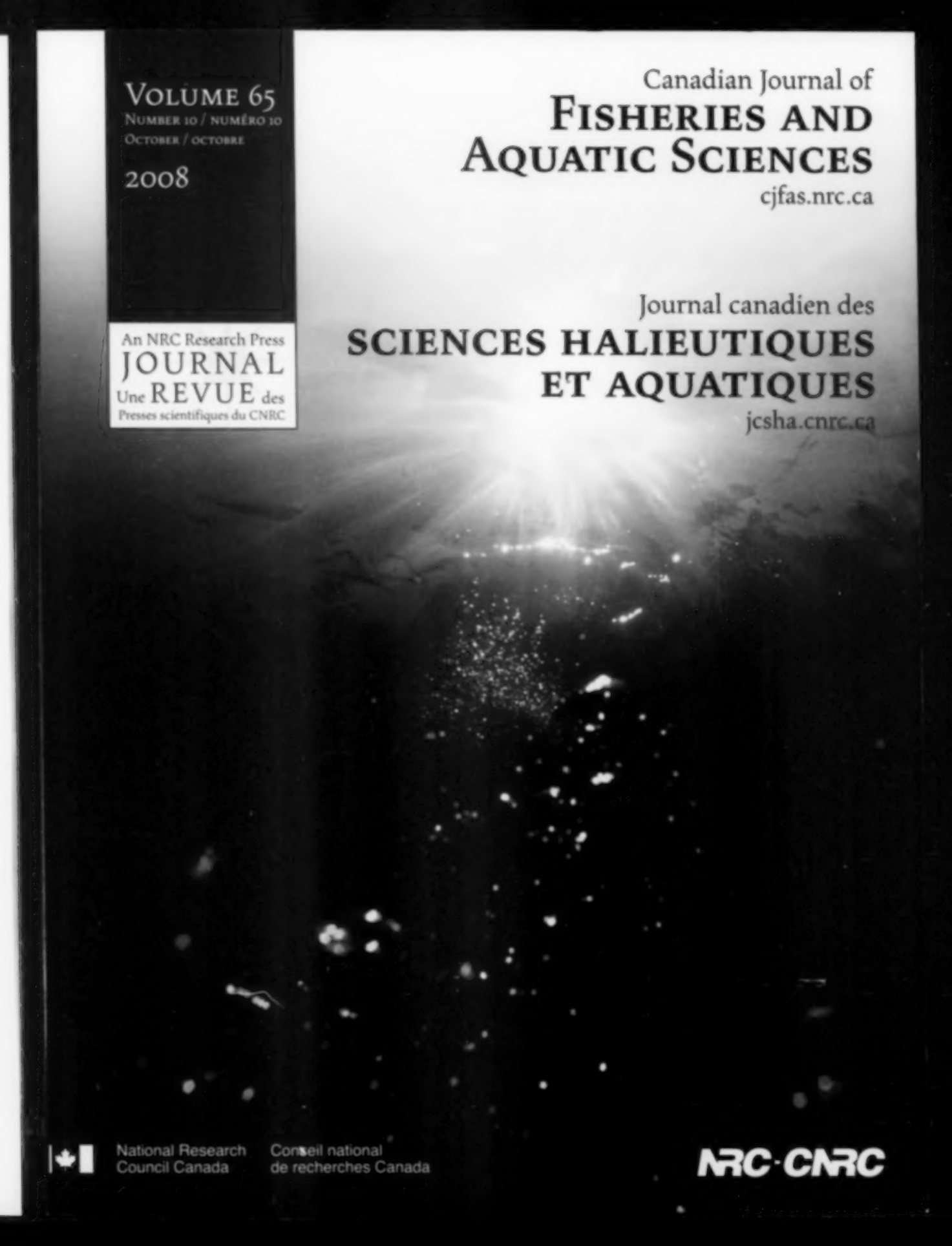

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The use of ventilation frequency as an accurate indicator of metabolic rate in juvenile Atlantic salmon (*Salmo salar*)

K.J. Millidine, N.B. Metcalfe, and J.D. Armstrong

Abstract: Bioenergetics studies of free-living animals have long been hampered by limitations on our abilities to measure the energy costs of different activities. Here we evaluate whether it is possible to use the opercular ventilatory beat rate of a fish to estimate its rate of energy expenditure. Changes in metabolic rate (MR) and ventilation rate (VR) were recorded in yearling Atlantic salmon (*Salmo salar*, weight range 1.8–12.64 g) engaged in different activities at different temperatures while within a respirometer. MR was found to correlate strongly with VR in all fish. The relationship was linear, and both the slope and corresponding intercept of the regression equation were strongly dependent on the fish's body weight and the test temperature. From these relationships, a general equation was generated to predict MR at a range of temperatures from knowledge of a fish's weight and its VR; this proved to be highly accurate (correlation between predicted and observed MRs: $r = 0.95$), although calibration of individual fish is recommended in studies that compare performance of individuals. Visual measurements of VR may therefore provide a highly accurate, cheap, and noninvasive method of measuring the energy consumption of fish engaged in natural behaviours in more natural settings.

Résumé : Les études bioénergétiques d'animaux en liberté ont longtemps été restreintes par l'impossibilité de mesurer les coûts énergétiques des différentes activités. Nous vérifions ici s'il est possible d'utiliser le taux de battement de ventilation des opercules d'un poisson pour mesurer son taux de dépense d'énergie. Nous avons enregistré dans un respiromètre les changements de taux métabolique (MR) et de taux de ventilation (VR) de saumons atlantiques (*Salmo salar*, étendue des masses 1,8 – 12,64 g) d'un an durant diverses activités à différentes températures. Il y a une forte corrélation entre MR et VR chez tous les poissons. La relation est linéaire et la pente et l'ordonnée à l'origine correspondante de l'équation de régression sont toutes deux fortement dépendantes de la masse corporelle du poisson et de la température expérimentale. À partir de ces relations, nous avons élaboré une équation générale pour prédire MR sur une gamme de températures d'après la masse du poisson et son VR. L'équation est très précise (corrélation entre les MRs prédits et observés : $r = 0,95$), bien nous recommandions de calibrer chacun des poissons lors d'études qui comparent les performances individuelles. Les mesures visuelles de VR peuvent ainsi constituer une méthode très précise, peu coûteuse et non effractive de déterminer la consommation d'énergie chez des poissons en train d'accomplir des comportements normaux dans des environnements plus naturels.

[Traduit par la Rédaction]

Introduction

All animal activity incurs an energy cost. There is major variation (both between and within species) in the extent of such costs, and this variation has great ecological importance. For instance, there are links between energy efficiency and life history patterns (Hayes et al. 1992), and metabolic costs can drive evolution (Mueller and Diamond 2001). However, limitations in our abilities to measure metabolic rates (MR) are still a methodological constraint in studies of ecological energetics. Various techniques have been used to estimate the energy expenditure of free-living animals (Grantner and Taborsky 1998). The use of doubly labelled water, for example, is suitable for measuring medium-term averages of energy expenditure of terrestrial animals (Speakman 1997). Treadmill experiments have also been used to estimate aspects of MR, including the locomotor capacity and costs of foraging behaviour of lizards (Huey et al. 1984; Garland et al. 1987) and thermoregulation during fasting in king penguins (*Aptenodytes patagonicus*) (Fahlman et al. 2005).

Estimates of the metabolism of free-ranging fish are difficult to obtain. While measuring MR by the oxygen consumption rate of single fish in a static or swimming respirometer is straightforward (Cech 1990), this method, with rare exceptions (Grantner and Taborsky 1998), does

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not allow measurements to be made on individual fish engaged in social behaviours on a scale of those living in more natural environments. Moreover, the doubly labelled water technique usually used to study free-living terrestrial animals is unsuitable because of the high water turnover rates of fish (Brodeur et al. 2001); the radioisotope tritium has a half-life of just 15 min, since it is quickly flushed out of the body via the gills (cited in Speakman 1997). Other approaches, such as bioenergetic modelling (Boisclair and Leggett 1989; Boisclair and Tang 1993; Rennie et al. 2005), can provide averaged values for energy costs over time scales of weeks or days, but are rarely possible over shorter time scales.

At present, one of the few ways of making estimates of MR in fish over time scales of minutes in the field is through telemetry of physiological correlates of MR such as ventilation rate (VR) (Oswald 1978; Rogers and Weatherley 1983; Dalla Valle et al. 2003), tailbeat frequency (Young et al. 1972; Stasko and Horrall 1976), locomotor muscle electromyograms (EMGs) (Briggs and Post 1997; Økland et al. 1997; Thorstad et al. 2000), and heart rate (Priede and Young 1977; Armstrong 1986; Lucas et al. 1991). While these techniques can work well, their use in small fish is limited, since they rely on the use of potentially invasive monitoring devices (which may impede small fish and so affect their energetic costs); they are also expensive and can be difficult to calibrate (Thorarensen et al. 1996). Furthermore, only some components of MR may be monitored. For instance, EMG telemetry can be used to estimate the direct cost of some activities, but can not measure the energy costs of feeding and assimilation of food or environmental stress. Therefore, to gain a fuller picture of the energy expenditure of fish in their natural environment, there is a need to develop new methods for estimating MR of free-living fish. One possibility is to use measurements of VR directly as a possible predictor of MR; this approach has been explored in several studies in the past (e.g., Van Rooij and Videler 1996; Grantner and Taborsky 1998; Dalla Valle et al. 2003), but these have not evaluated its reliability under a broad range of situations.

The objective of the present study was therefore to determine whether VR is sufficiently accurately related to MR in juvenile Atlantic salmon *Salmo salar* at a range of temperatures within their normal environmental range and over a range of different activities so as to allow energy expenditure to be predicted from observations of VR.

Materials and methods

The experiments were carried out on yearling hatchery-reared Atlantic salmon parr derived from wild parents; these were reared at the Fisheries Research Services Almondbank field station, Perthshire, and transferred to Glasgow University at the end of April 2006, where they were held in a circular tank (1 m^2) at 8 °C in aerated, recirculated, copper-free water under an ambient photoperiod. They were fed to satiation on defrosted bloodworms (Chironomid larvae) once a day. While in the holding tank, the fish had access to shelters in the form of large stones and lengths of semicircular cut piping (approximately 120 mm in diameter). On 7 May 2006, 12 of these fish were selected at random (mean body mass 3.04 g ± 0.83 g standard deviation, SD, range 1.76–4.50 g) and transferred to individual holding tanks under the same environmental conditions as in the holding tanks. They were allowed to settle for 2 weeks before the first respirometry experiments commenced. These fish were used throughout the experiment, which involved simultaneous measurements of MR and VR during different activities at a range of temperatures (8, 13, and 16 °C); use of the same fish at different temperatures allowed us a more accurate analysis of the effect of temperature than if separate fish were used.

In each run, four fish were placed individually without food in four separate respirometer chambers, each of which contained a clear perspex shelter, since fish have a higher resting rate of metabolism when denied access to any shelter (Millidine et al. 2006). The apparatus was similar to that described by Millidine et al. (2006) and allowed measurements of oxygen consumption to be made using intermittent flow respirometry based on a Strathkelvin Instruments SI130 oxygen electrode linked to a SI928 6-channel meter. VR was recorded as the number of opercular beats per 20 s (but expressed as beats·min^{-1} in analyses), with the fish being observed from the side through a slit in the screens that otherwise enclosed the tank.

Each fish was retained within the respirometer for 5 days to measure both MR and VR over a range of activities (initial settling, resting immobile, feeding–digestion, responding to disturbance) that would generate significant variation in MR. Measurements were made more frequently at times when MR was changing more rapidly (e.g., when settling, after disturbance, and immediately after having been fed), so that a roughly equal distribution of measurements was obtained across the spectrum of MR. The basic protocol was as follows (further details of each stage being given further below). The first 2 days constituted the settling period, during which the MR of the fish dropped from an initially high level (owing to the stress of being handled and placed in a new environment) down to a stable, resting level. This was also a period of initial exploration, after which fish were generally inactive and resting on the bottom of the chamber (either under or adjacent to the shelter) for the remainder of the trial. Each fish was then fed on the third day and its apparent specific dynamic action (SDA) was recorded. On the fifth day, the fish were deliberately disturbed, before being removed from the respirometer and returned to their individual flow-through tanks. This procedure was then repeated in the following run using the next group of four fish. Once all 12 fish had been tested at the first temperature (8 °C), the temperature in holding and experimental tanks was increased to 13 °C. The fish were allowed to acclimatize to the new temperature for 2 weeks before the trials were repeated so that each fish experienced settling, feeding, and disturbance. When the trials had finished at this temperature, the procedure was repeated at 8 °C again (to check for sequence or temporal effects and to allow cross-validation of the predictive equations — see below) and then gradually to 16 °C (at a rate allowing for acclimatisation; Wright et al. 2001). The fish grew over the time period of the experiment (140 days; mean gain in mass 183% ± 166% SD, range 44%–618%), so providing a robust test of the effects of fish size on the VR–MR re-

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lationship. Blank runs were also carried out after each week (before new fish were put back in) to measure the biological oxygen demand; the system was closed for a period of 1 h and the drop in percent saturation of oxygen was recorded (biological oxygen demand < 0.038 ml $O_2 \cdot h^{-1}$ in all cases and therefore assumed to be negligible).

Settling period

Fish were placed into the respirometer chambers between 0800 and 0900 on day 1. Both MR and VR were measured as soon as each fish was introduced to the respirometer and continued at regular intervals for a further 8 h that same day and again between 0900 and 1700 the following day. By this time the MR of all fish had dropped to a stable level, taken to be standard MR (after the initial period of introduction, fish spent virtually all their time resting motionless on the bottom of the respirometry chamber either in or just next to the shelter). MR was measured from the reduction in water oxygen concentration over a period of 6 min, when the water flow-through system was temporarily closed, and this procedure was repeated at intervals of 15 min for the first hour after introduction of the fish to the respirometer and then every 30–35 min throughout the rest of day 1. On day 2, the MR was measured a further three–four times. VR was recorded for 20 s every minute throughout the period of measurement of MR and then averaged.

Feeding and SDA

Food, in the form of defrosted bloodworms, was given to the fish while inside the respirometers. Since our aim was to ascertain whether the relationship between MR and VR still existed while the fish were obtaining and digesting food, the exact amount of food given to each individual was not important provided it gave a measurable increase in SDA. As soon as the bloodworms were introduced into the respirometer, the system was closed for a period of 6 min and the MR and VR were measured. Then the system was re-opened, and when the water within the respirometer was completely flushed with 100% saturated water (approximately 3 min), the system was again closed for a further 6 min. This process was continued until the change in MR from one period of closure to the next was much reduced (<10% change). When this point had been reached, the system was closed for 6 min every 30–35 min for the remainder of the day and for a further three–four times through the following day (0900–1700), by which time the MR in all cases had dropped to a level very similar to the original standard MR (mean % difference = 8.65 ± 0.07 standard error, SE).

Disturbance

To create a disturbance, the lid of the respirometer was removed and a blunt instrument was inserted into the water and gently moved around to elicit a swimming response by the fish. After about 10 s, the lid was replaced and the oxygen consumption and VR were measured for periods of 6 min immediately after disturbance and 36 min later. This disturbance procedure was carried out three separate times on each fish at each temperature. The fish were then removed from the respirometers and returned to their individual holding tanks, where they remained until they were tested again at the next temperature.

Analysis

MR was plotted against VR for each fish and temperature, combining all the data from a single 5-day trial (i.e., settling, feeding, and disturbance measurements) so as to determine the relationship between VR and MR over the broadest possible range of MRs for that individual at that body size and temperature. Lognormal and power fits were tested but rejected in favour of normal linear fits, which explained the greatest proportion of variation. Since the relationships were linear, it was then possible to test whether both the slope and the intercept of the VR–MR regression lines were dependent on (and so could be predicted from) fish size and temperature. We ran two general linear mixed model (GLM) analyses, with the observed values for either slope or intercept (taken from all available fish at both 13 and 16 °C, but a randomly selected half of the fish from the first 8 °C trial and the other half from the second 8 °C) being the dependent variable, and temperature, body weight, and the interaction between weight and temperature as candidate independent variables; both untransformed and logarithmic values of body weight were used. Fish identity was included as a random factor to control for the nonindependence of data points from the same individual. The analyses were run by repeatedly eliminating the least significant term until only significant terms remained.

Results

There was a significant linear relationship between VR and MR in all tested cases. However, because of a small number of mortalities during the 5-month experimental period, not all fish were tested on each of the four occasions (overall, eight fish were tested four times, so each generated three regression plots after elimination of one 8 °C trial, two fish were not tested at 16 °C, and two fish were only tested at a single temperature, producing a total of 30 regression plots; n = 18–27 data points per plot; in all cases $r > 0.80$, $P < 0.001$, significant after sequential Bonferroni correction (Rice 1989)). Example plots are given for two fish showing the relationship between MR and VR over the ranges of temperatures and activities (Fig. 1).

The GLM analysis showed that the slope of the regression predicting MR from VR could be predicted from knowledge of fish weight (W, in g), temperature (T, in °C), and the interaction between these two explanatory variables (Table 1, part (*i*) and eq. 1; Akaike's information criterion (AIC) = −149.56):

$$(1)\quad \text{Slope} = 0.2773 - [0.2350 \times \log_{10}(W)] - (0.01838\ T) + [0.05813\ T \times \log_{10}(W)]$$

The intercept of the MR–VR regression could similarly be predicted from these variables, with the addition of the (untransformed) body weight (Table 1, part (*ii*) and eq. 2; AIC = 35.68):

$$(2)\quad \text{Intercept} = -3.4078 + 0.2958\ T + [2.1956 \times \log_{10}(W)] - [0.82057\ T \times \log_{10}(W)] + 0.5335\ W$$

Equations 1 and 2 were then used to formulate a general relationship to predict MR from VR (MR = m(VR) + c), in

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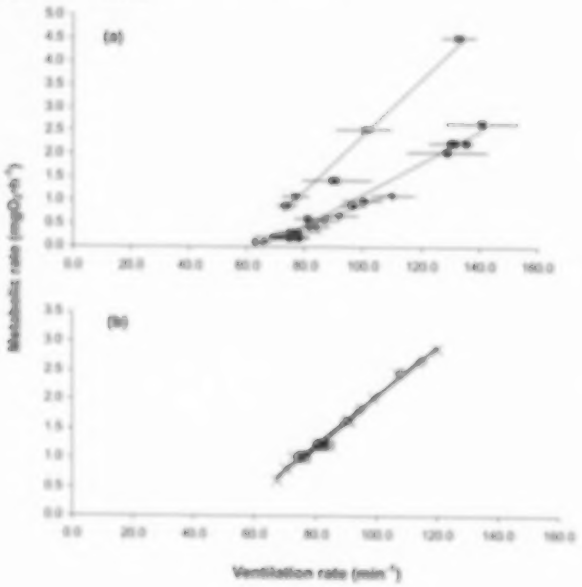
Fig. 1. Examples of the relationship between metabolic rate (MR) and ventilation rate (VR). (*a*) The effect of temperature (diamonds = 8 °C, squares = 13 °C, crosses = 16 °C). The same salmon was used at all temperatures and weighed 2.15 g when tested at 8 °C (r^2 = 0.97), 2.98 g at 13 °C (r^2 = 0.99), and 5.66 g at 16 °C (r^2 = 0.98). Each data point shows the mean VR ± standard deviation. (*b*) The effect of activity at a single temperature (13 °C: crosses = settling (r^2 = 0.99), diamonds = feeding (r^2 = 0.99), squares = disturbance (r^2 = 0.94). The salmon weighed 5.94 g (combined r^2 across all activities = 0.99).



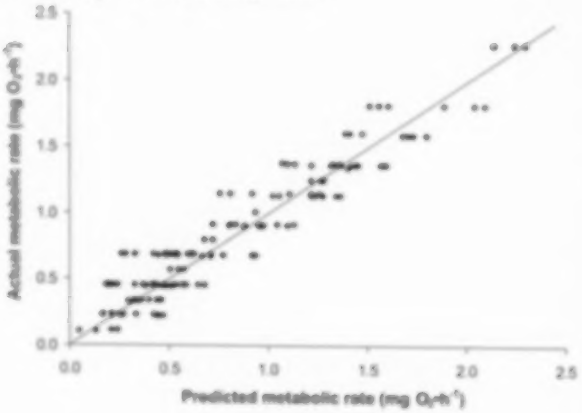
which VR is expressed as beats·min^{-1}, m is derived from eq. 1, and c is from eq. 2. The accuracy of this equation was evaluated by cross-validation, comparing the values for MR observed with those predicted using the remaining half of the 8 °C results (those not used to generate the equations). This was carried out by calculating the mean observed and predicted MR in each activity (settling, feeding, and disturbance) for each fish and then running a repeated measures analysis of variance (ANOVA) with activity (three levels) and the measurement type (observed or predicted MR; two levels) as the within-subjects factors and fish weight as a covariate. The observed values were not significantly different from those predicted (measurement type: $F_{[1,8]}$ = 0.375, P = 0.56) and showed a close general agreement across all rates of energy expenditure (Fig. 2). There was no effect of activity type or fish body weight on the discrepancy between observed and predicted MR (measurement type by activity interaction: $F_{[2,16]}$ = 1.025, P = 0.381; measurement type by weight interaction: $F_{[1,8]}$ = 0.483, P = 0.51), indicating that the predictive power of the equations was not influenced by the type of activity occurring at the time of the measurements (i.e., whether the fish was settling, digesting food, or disturbed) nor by the size of the fish. However, deviation from unity varied among fish. When fish identity was included as a factor in a GLM that analysed the data in Fig. 2, it explained some of the variation in observed MR (effect of fish identity: $F_{[9,101]}$ = 57.07, P < 0.001; effect of predicted MR: $F_{[1,101]}$ = 3531.86, P < 0.001; fish identity by predicted MR interaction: $F_{[9,101]}$ = 1.53; P = 0.14). Thus the predicted–observed regression line differed slightly in elevation among fish, although there was no significant difference in the slopes of those lines.

Table 1. Results of general linear mixed model analysis to test whether the slope and intercept of the regression of metabolic rate on ventilation rate could be predicted from knowledge of the weight (W) of fish and the temperature (T).

Independent variables	Unstandardized regression coefficients	t	P
***(i)* Slopes**			
Constant	0.2773	3.700	0.001
$\log_{10}(W)$	–0.2350	–2.109	0.045
$\log_{10}(W)\,T$	0.0581	6.678	<0.001
T	–0.0184	–2.843	0.009
***(ii)* Intercepts**			
Constant	–3.408	–3.618	0.001
T	0.296	3.036	0.006
$\log_{10}(W)$	2.196	1.615	0.119
$\log_{10}(W)\,T$	–0.821	–5.853	<0.001
W	0.534	3.730	0.001

Note: Observed values for (*i*) slopes and (*ii*) intercepts are used as the dependent variable; significant predictor variables are listed in the text (in (*i*), the untransformed body weight and its interaction with temperature were not significant and were removed from the model; $\log_{10}(W)$ was also not significant in (*ii*) but was retained in the model, since $\log_{10}(W)\,T$ was significant).

Fig. 2. The relationship between metabolic rate (mg O_2·h^{-1}) as predicted using the regression equations and the fish's actual metabolic rate (mg O_2·h^{-1}). The data shown here were not used to generate the predictive equations. Overall r^2 = 0.91; the slope of a regression line (0.89 + 0.11 ± 0.018 standard error) is not significantly different from a line of equality (shown here), indicating no systematic departure between predicted and observed values over the documented range of rates of metabolism.



Discussion

The objective of this study was to determine whether VR

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of juvenile Atlantic salmon was a good correlate of MR during settling, feeding, and disturbance periods, across a range of temperatures. If VR is to be a reliable predictor of MR outside the respirometer, it needs to satisfy certain criteria. First, it must have a close mathematical relationship with MR over a range of both activities and temperatures. Second, it must be possible to use any such relationship to create a general equation that can accurately predict the MR of fish in a range of environmental conditions and so provide a valuable tool to investigate the bioenergetics of fish in more natural settings than the solitary confinement of small respirometers. Third, ideally a common relationship between VR and MR should exist at the population level so that there is no need for extensive calibration of fish. This study showed that in salmon, the relationship between VR and MR was well described by a very tight linear fit across a range of activities at any given temperature; fish naturally had much higher rates of oxygen consumption in some contexts than others, but the cause of an elevation in metabolism (e.g., whether due to recently being disturbed or to eating and assimilating a meal) did not affect the manner in which MR was related to VR. Moreover, while the position of the regression lines varied with both the body weight of the fish and the temperature, in both cases the patterns were statistically predictable. Thus the slope of the regression line increased with increasing temperature and body mass (i.e., a given VR corresponded to a higher MR at higher temperatures and in larger fish), whereas its corresponding intercept tended to decrease with increasing temperature and body mass. There was a high correlation between the MR predicted from these variables and the actual MR as measured by the respirometer ($r = 0.95$). However, there was nevertheless sufficient, unexplained, among-individual variation to generate significant scatter in predictions of MR. Therefore, although the general equations are good for estimating absolute MR at the population level and change in MR within individual salmon, individual calibration may be required for comparison of individuals within populations.

In principle, changes in MR can be accommodated by a change in VR, buccal volume, or an increased efficiency of oxygen extraction from water passing the gills; to an extent, alterations in MR may be independent of the ventilatory system if cutaneous uptake is important. The percent contribution of VR to change in oxygen consumption (%conVR) at a given weight and temperature is given by $(\log VR_2 - \log VR_1)/(\log MR_2 - \log MR_1) \times 100$, where subscripts 1 and 2 denote minimum and maximum values for the parameter, respectively, across a measurement range (Scharold and Gruber 1991; Armstrong and Smith 1992). The %conVR for salmon parr was 27.7% (8 °C), 31.6% (13 °C), and 32.4% (16 °C); therefore, there is clearly a substantial contribution of buccal volume (e.g., Kiceniuk and Jones 1977; Altimiras and Larsen 2000; Dalla Valle et al. 2003) and (or) variation in other respiratory parameters as MR varies. However, the good approximation of a linear relationship between VR and MR and small variation around the relationship indicates that changes in VR are closely coupled and proportional to overall changes in gill stroke volume and other potential variables. Change in buccal volume and other respiratory parameters as fish grow and temperature varies would be expected to influence the patterns of variation in MR–VR relationships across fish weight and thermal regimes. Similarly, variations in these parameters may account for some of the residual among-individual variation in MR–VR relationships beyond that attributed to body mass and temperature.

Several previous studies have found close relationships between VR and MR of fish, but such studies have each considered only a limited range of temperatures, behaviours, or time scales, and so the broad applicability of the method has been unknown. Thus, while Van Rooij and Videler (1996) found that MR was closely related to VR across a range of body sizes in the reef fish *Sparisoma viride*, they did not evaluate the effect of water temperature nor varying activity patterns. Grantner and Taborsky (1998) looked at VR and MR in the cichlid fish *Neolamprologus pulcher*. Mean VR was shown to correlate significantly with routine MR ($r^2 = 0.32$) and with MR when more active behaviours were exhibited ($r^2 = 0.66$); the relatively low variance in MR explained by VR in their study in comparison with our own may be due to the fact that their analysis did not control tightly for temperature or body weight. Dalla Valle et al.'s (2003) study of VR in flounders is similarly restrictive, since they made measurements only over a 2-day period as the fish were recovering from surgery. To our knowledge, our study is the first to evaluate the method over a sufficiently broad range of environmental and behavioural situations to reveal its true potential and is the first to produce equations that permit the prediction of MR from opercular beat rates.

An advantage of using visual recording of VR in preference to some other approaches is that it is noninvasive. Although radio and acoustic telemetry-based measurements of opercular or heart muscle contractions potentially allows the automated monitoring of metabolism in wild fish, in practice the method is restricted to relatively large individuals and is limited by the duration of the transmitter (Rogers et al. 1981; Weatherley et al. 1982; Lucas et al. 1991). The value of heart rate as a measure of MR varies among species of fish (Armstrong et al. 1992), and the relationship between these variables may differ as a function of environment and with time after disturbance (Thorarensen et al. 1996; Webber et al. 1998). For salmon parr, the relationship between VR and MR identified in the present study appears to be substantially more stable than that recorded between heart rate and metabolism in small salmonids (Priede and Tytler 1977; Thorarensen et al. 1996). However, further calibration would be wise should VR be applied as a measure of metabolism under unusual conditions, for example, in severely hypoxic and polluted waters.

The results obtained here open up the possibilities of estimating MR in contexts that have previously been very difficult to obtain, such as fish engaging in social interactions in aggregations or performing activities more complex than simply swimming in straight lines or eating a single meal. Although the method relies on being able to observe the fish without disturbing it, the development of both large-scale, controlled environment streams (MacLean et al. 2005) and underwater, remotely operated video equipment should permit the energy expenditure of salmon parr across a broad range of activities to be measured for the first time in natural and large-scale near-natural conditions.

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Impacts of environmental change and direct and indirect harvesting effects on the dynamics of a marine fish community

Hugues P. Benoît and Douglas P. Swain

Abstract: We describe dramatic shifts in the species composition of the marine fish community of the southern Gulf of St. Lawrence using a 35-year time series of catch rates in an annual bottom-trawl survey. We attempt to understand the causes of these changes using a traits-based approach that relates the similarity among species in their abundance trends to similarities in their ecological traits. We selected traits based on a priori beliefs of how each should reflect susceptibility to changes in a different external factor potentially affecting the community. We found evidence for an effect of ocean climate and top-down effects of fishing and seal predation, but not for bottom-up effects of prey availability on adult fishes. Mean body length in the community decreased dramatically in the 1990s. This reflected the removal of large-bodied fishes by fishing and sharp increases in the abundance of small fishes. The biomass of small fish was inversely correlated with an index of predation on those fish by larger fish, suggesting strong predator control of the abundance of small-bodied fishes. Our results suggest that changes in ocean climate combined with direct and indirect effects of harvesting can dramatically and rapidly alter the composition of marine fish communities.

Résumé : Nous décrivons des changements importants de composition spécifique dans les communautés de poissons marins du sud du golfe du Saint-Laurent à partir d'une série chronologique de 35 années de taux de captures dans des inventaires annuels au chalut de fond. Nous tentons de comprendre les causes de ces changements à l'aide d'une méthodologie basée sur les traits qui met en rapport les similarités des tendances d'abondance chez les espèces et les similarités de leurs traits écologiques. Nous avons choisi les traits d'après notre perception a priori de comment chaque trait devrait refléter la susceptibilité aux changements d'un facteur externe particulier qui affecte potentiellement la communauté. Nous trouvons des indications de l'existence d'un effet du climat océanique et d'effets descendants de la pêche et de la prédation par les phoques, mais non d'effets ascendants de la disponibilité des proies sur les poissons adultes. La longueur corporelle moyenne dans la communauté a diminué de façon importante durant les années 1990. Cela reflète le retrait des poissons de grande taille par la pêche et un accroissement marqué des petits poissons. Il existe une corrélation inverse entre la biomasse des petits poissons et un indice de prédation des gros poissons sur les petits, ce qui laisse croire à un important contrôle de l'abondance des poissons de petite taille par les prédateurs. Nos résultats indiquent que les changements dans le climat océanique combinés aux effets directs et indirects de la récolte peuvent modifier de façons importante et rapide la composition des communautés de poissons marins.

[Traduit par la Rédaction]

Introduction

There is increasing recognition of the need to consider fisheries in the context of the ecological communities and ecosystems in which they are prosecuted (e.g., Link 2002; Pikitch et al. 2004; Mangel and Levin 2005). Because of interspecific interactions, responses to harvesting may be more complex than those predicted by single-population models (e.g., Sih et al. 1985; Yodzis 2001; Bascompte et al. 2005). Furthermore, separating projected responses to harvesting (including top predator culling) from those caused by changes in climate (e.g., Bennett et al. 2004; Rose 2004) is crucial, because of these two effects, only the human activities related to harvesting can realistically be managed on the temporal and spatial scales that are relevant to stock and community dynamics. Retrospective analyses and modelling of exploited marine communities founded in multiple a priori hypotheses are necessary to understand such species and community responses (Hilborn and Mangel 1997).

Changes in the composition of marine fish communities have been reported for a number shelf ecosystems in recent years (e.g., Fogarty and Murawski 1998; Greenstreet et al. 1999; Choi et al. 2004). The top-down effects of fishing (e.g., Bianchi et al. 2000; Link and Garrison 2002; Frank et al. 2005) and effects of climate change (e.g., Perry et al. 2005; Poulard and Blanchard 2005) are often cited as principal causal factors. Quantitatively assessing the relative influence of these and other factors remains a particular challenge, though canonical multivariate analyses and approaches based on aggregate community properties (e.g., size composition, species richness, and diversity) have

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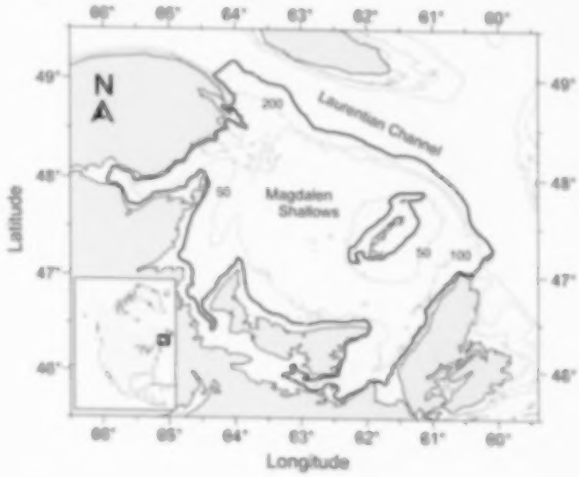
shown some promising results (e.g., Link et al. 2002; Blanchard et al. 2005; Worm et al. 2005).

In this paper, we describe profound changes from 1971 to 2005 in the species composition of the marine fish community in the southern Gulf of St. Lawrence (Northwest Atlantic, Canada; Fig. 1). We examine five competing hypotheses for the factors driving these changes: (*i*) direct top-down effects fishing; (*ii*) changes in predation by a marine-mammal top predator, the grey seal (*Halichoerus grypus*); (*iii*) climate variation; (*iv*) bottom-up effects on adult fishes of changes in the availability of their prey; and (*v*) changes in predation by marine fishes (i.e., release of small fish from predation as large fish are depleted by fishing, an indirect effect of fishing).

These hypotheses were selected based on observed changes in the underlying forcing factors and (or) their proposed importance in single species dynamics or other ecosystems. Overfishing has been implicated as the principal cause of the collapse of Atlantic cod (*Gadus morhua*) and other exploited groundfishes in the Northwest Atlantic (e.g., Myers et al. 1996; Sinclair and Murawski 1997). Fishing effort (and thus fishing mortality) in the southern Gulf was high throughout the 1970s and 1980s, rose to very high levels in the late 1980s and early 1990s, and has been at a low level since 1993 when a moratorium on directed fishing for cod was initiated (Fig. 2*a*). The abundance of grey seals in this and neighboring areas has been increasing exponentially for over three decades (Fig. 2*b*) (Bowen et al. 2003) and is correlated with high levels of natural mortality in the local cod population (Chouinard et al. 2005). Mean bottom-water temperatures cooled throughout the 1980s, resulting in a period of record-low temperatures in the early to mid-1990s (Fig. 2*c*) (Gilbert and Pettigrew 1997; Drinkwater and Gilbert 2004); comparable change in a neighboring area has been linked with apparent shifts in species distributions (Frank et al. 1996). Bottom-up effects of changes in prey availability have been observed in other systems (e.g. Beaugrand et al. 2003; Frederiksen et al. 2006) and may be important in our study area given observed long-term changes in the diet of southern Gulf cod (Hanson and Chouinard 2002). Finally, dramatic increases in the abundance of forage fishes in other ecosystems have been attributed to reduced predation following declines in the abundance of large piscivorous fishes (e.g., Daan et al. 2005; Frank et al. 2005; Ward and Myers 2005).

We examined the hypotheses for the causes of community change using a traits-based approach that relates the similarity between species in their population dynamics (i.e., their abundance trends) to the similarity in their ecological and functional traits. Analogous trait-based approaches have been used to successfully predict changes in species composition of a plant community during secondary succession (Shipley et al. 2006) and have been advocated as a promising avenue towards predictive community ecology (McGill et al. 2006). This approach is predicated on the idea that a given environment selects or filters for optimal states across a suite of traits, and species with trait combinations close to the optimum are favored (e.g., Keddy 1992). Changes in the environment (including modifications due to biological interactions and fishing) select for different trait values and lead to changes in the relative abundance of species. Synchronous changes in abundance of a group of species sharing certain traits (e.g., cold-water tolerance) therefore imply a common response to particular driving factors (e.g., changes in environmental temperatures).

Fig. 1. Southern Gulf of St. Lawrence bottom-trawl survey area (dark line) with 50, 100, and 200 m isobaths (grey lines). The survey covers depths from 15 to 350 m. The inset map shows the study location in North America.



To examine our five competing hypotheses for the causes of fish community change in the southern Gulf of St. Lawrence, we attempted to select traits believed a priori to reflect susceptibility to the forcing factor underlying each hypothesis. We were able to identify an appropriate trait for four of the five hypotheses. These traits were (*i*) relative fishing mortality, reflecting direct fishing effects; (*ii*) an index of susceptibility to predation by grey seals; (*iii*) thermal preferences (as inferred from biogeographic distribution), believed to reflect susceptibility to climate variation; and (*iv*) diet composition, believed to reflect susceptibility to bottom-up effects of variation in prey availability. We were unable to identify a trait that unambiguously reflected susceptibility to changes in predation by marine fishes. Thus, instead of a traits-based approach, the fifth hypothesis was examined using the traditional approach of correlation, relating a response variable (biomass of small fish) to a potential forcing factor (an index of fish predation on small fish). These analyses provide evidence for effects of climate variation and top-down effects (i.e., consumer control) of fishing and predation on the composition of the marine fish community in the southern Gulf but fail to provide evidence of bottom-up effects of variation in prey availability on adult fishes (i.e., resource control).

Materials and methods

Changes in species composition of the fish community

Changes in species composition of the fish community in the southern Gulf of St. Lawrence were described based on catch rates (mean number of fish per standard tow) in a bottom-trawl survey conducted each September since 1971

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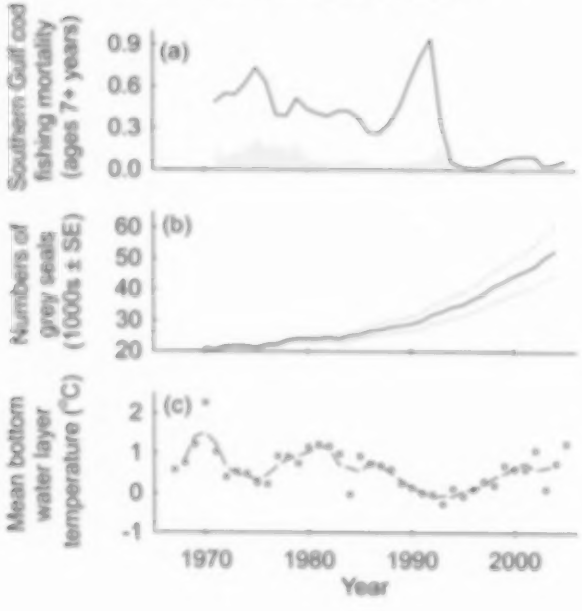
Fig. 2. Temporal trends in three hypothesized causal factors for the changes in species composition in the southern Gulf of St. Lawrence. (*a*) Mobile (open area below line) and fixed-gear (shaded area) fishing mortality of adult Atlantic cod (*Gadus morhua*) in the southern Gulf (1971–2005; from Chouinard et al. 2006). Cod fishing mortality is correlated with total groundfish fishing effort in the ecosystem ($r = 0.89$ for mobile gear and $r = 0.74$ for fixed gear) and is therefore likely a good proxy for the fishing mortality of other species captured across all groundfish fisheries. (*b*) Abundance of grey seals (*Halichoerus grypus*) in the Gulf of St. Lawrence herd, 1970–2005 (from Hammill 2005). (*c*) Mean water temperature (○) and 3-year running average (—), 1967–2005, in the southern Gulf of St. Lawrence cold intermediate layer, the water layer that covers most of the bottom in the area.



(Fig. 1; see Hurlbut and Clay 1990 for details on survey methodology). A 19 mm trawl cod-end liner was used in all years to retain small fish. Where necessary, catch rates were adjusted for changes in gear, vessel, or survey protocol, as described by Benoît and Swain (2003) and Benoît (2006*b*). For each species i and year j, the standardized catch rate, c_{ij}, was then transformed by taking $[c_{ij} - \min(c_i)] / [\max(c_i) - \min(c_i)]$, thereby reducing the values for each time series to the interval [0,1]. Only the 52 most abundant fish species were retained for analysis (Appendix A, Table A1). Owing to problems with taxonomic identification, six taxa were grouped at the level of genus or higher (Appendix A, Table A1; for explanation see Benoît and Swain 2003). These groups are nonetheless referred to commonly as "species" in the text for simplicity.

Temporal changes in species composition over the period 1971–2005 were summarized using a correspondence analysis (CA) of the $N \times Y$ catch rate matrix, $\mathbf{C}$ ($N = 52$ species, $Y = 35$ years). CA is a method of reduced-space ordination that is recommended when data contain a relatively large number of null values (Legendre and Legendre 1998). We used CA for two purposes. Species scores on the first CA eigenvector (CA-I) were used to sort species in a plot of $\mathbf{C}$, thereby highlighting the major pattern of temporal change in the community species composition. Secondly, a biplot of CA-I and CA-II was used to display changes in community trait composition (described below).

Species traits

Four traits were examined, each reflecting susceptibility to a forcing factor hypothesized to underlie the change in community composition. A separate matrix of species values, denoted $\mathbf{T}_t$, was created for each trait, t, as described below. The traits were exploitation level, susceptibility to predation by grey seals, biogeographic distribution, and diet.

Exploitation level was defined so as to reflect the average fishing mortality experienced by each species relative to their productivity. First, we calculated F_i, the 1971–2005 average relative fishing mortality for species i, adjusted by an index of productivity for that species:

$$(1) \qquad F_i = \frac{1}{35 \cdot m_i} \sum_{j=1971}^{2005} \frac{L_{ij} + D_{ij}}{B_{ij}}$$

where m_i is an estimate of the natural mortality rate for species i, L_{ij} is landed catch in year j, and D_{ij} and B_{ij} are annual estimates of total discarded catch and catchability-adjusted survey biomass, respectively. Catchability adjustments were based on length-dependent catchability models developed for a number of species and applied to species with generally similar body shapes and behaviour (see Appendix A, Table A1). Discarded catch was estimated using species-specific discard data collected by an at-sea fisheries observer program covering 5%–30% of commercial fishing trips since 1990 (see Benoît 2006*a* for details on the estimation). Natural mortality was included in the calculation because species with higher m tend to have higher production rates and can therefore sustain higher levels of fishing mortality (Beverton and Holt 1959). m was estimated based on an interspecific relationship between natural mortality rate and the Brody somatic growth coefficient, K ($m \approx 1.65K$; Charnov 1993). An estimate of K was available for 23 of the 35 species with nonzero fishery catches (Froese and Pauly 2007). For the remaining 12 species, K, which is known to be correlated with ultimate individual length (L_∞) from the von Bertalanffy growth equation, was estimated based on the maximum body length observed in the survey, L_{max}. Based on the species for which we had an estimate for K, $K = 0.3140 - 0.0017\ L_{max}$ ($R^2 = 0.36$, $n = 33$). Because of the large uncertainty in estimates of many of the quantities in eq. 1, we considered F_i to accurately reflect only order-of-magnitude differences in exploitation impacts. Thus, exploitation level was scored accordingly (Table 1), resulting in a semiquantitative ordinal trait.

A second trait examined was susceptibility to predation by grey seals. The grey seal is an important top predator of fishes in the community (Hammill and Stenson 2000). For each species i, we calculated S_i, an index of its susceptibility to predation by grey seals relative to its productivity:

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Table 1. Exploitation level score as a function of relative fishing mortality, F.

Score	F	General characteristics of species occurring in the category
1	>0.5	Targeted species with landings >75% of quota determined by stock assessment or species commonly captured in large quantities as bycatch
0.67	0.05–0.5	Targeted species with landings 25%–75% of quota determined by stock assessment or species frequently captured as bycatch
0.33	0.0005–0.05	Some capture of the species was reported or estimated, but quantities were small relative to their biomass in the survey
0	~0	Very few or no instances of capture reported

$$(2) \qquad S_i = d_i \left(\sum_{j=1990}^{2004} B_{ij}/15 \right)^{-1} m_i^{-1}$$

where m_i and B_{ij} are as defined above, and d_i is the proportion, by mass, of southern Gulf grey seal diets that are composed of species i from a study of digestive tract contents conducted over the 1990–2004 period (Hammill et al. 2007). Because of differential digestibility of prey items recovered from seals, imperfect characterization of spatial and seasonal differences in diet composition, and the uncertainties regarding m_i and B_i, S_i was transformed into a semi-quantitative trait scored as 0 (apparently not consumed), 0.5 (below median S_i), or 1 (above median S_i). Skates, which lack the bony parts used to infer seal diets, were scored as 1 based on observed consumption in other areas (Benoît and Bowen 1990). Other species not reported in seal diets observed in the southern Gulf but occurring in diets for neighbouring areas (also reported in Hammill et al. 2007) were scored as 0.5.

Biogeographic distribution was a third trait we examined and was expected a priori to reflect thermal preferenda and therefore the response to changes in temperature. It was composed of a set of three categories consisting of the proportions of the species' west Atlantic geographic range occurring in each of three zones: (1) Arctic–subarctic (north of 51 N), (2) boreal (between 44 N and 51 N), and (3) temperate (south of 44 N). These proportions were estimated based on a qualitative assessment of general accounts (Scott and Scott 1988; Brown et al. 1996; Colette and Klein-MacPhee 2002; Froese and Pauly 2007).

The fourth trait examined was diet. It was also composed of a set of categories consisting of the proportions of the diet by weight in each of eight prey groups: (1) fish eggs and larvae, (2) adult fish and squid, (3) marine worms, (4) shrimps and crabs, (5) plankton, (6) mollusks other than squid, (7) echinoderms, (8) other prey. Proportions were based on published data (Bowman et al. 2000; Hanson and Chouinard 2002; Savenkoff et al. 2004). Where size-specific data were available, the diet of an average-sized individual for the survey (generally adult fish) was used.

For the six taxa grouped to a level greater than species in the analysis (Appendix A, Table A1), the traits of the most common species in the group were used. Species trait values are presented graphically in Fig. 3.

Relating dissimilarity in abundance trends to species traits

Distance-based redundancy analysis (dbRDA; McArdle and Anderson 2001), a form of multivariate multiple regression, was used to assess the relative importance of each of the traits (explanatory variables), $\mathbf{T}_i$, in explaining differences among species in their time trends of survey abundance (response variables). dbRDA is performed directly on a distance or dissimilarity matrix, $\mathbf{D}_\mathbf{C}$, a triangular matrix of pairwise measures of the dissimilarities in time trends between the species in $\mathbf{C}$ in our case. We calculated the dissimilarity in abundance trends using χ^2 distance. (Results were similar using another commonly used distance measure, Bray–Curtis dissimilarity).

The importance of each trait was first assessed individually in marginal tests. Then a forward selection procedure was used to find the suite of traits that best explained the dissimilarity among species in their abundance trends. Finally, the conditional (partial) significance and effect size of each of the selected traits was evaluated by testing the effect of one trait while controlling for effects of the others. Colinearity between traits was assessed using pairwise Mantel correlations (Legendre and Legendre 1998) of individual trait dissimilarity matrices, $\mathbf{D}_\mathbf{T}$. The χ^2 distance was used to calculate $\mathbf{D}_\mathbf{T}$ for the traits biogeographic distribution and diet, and absolute arithmetic difference was used for the other two traits.

Statistical significance in the dbRDA was assessed using 9999 unrestricted but simultaneous permutations of the rows and columns of $\mathbf{D}_\mathbf{C}$ in the case of marginal tests and of the multivariate residual matrix in the case of conditional tests (Anderson 2003; Anderson and Legendre 1999). All dbRDAs were performed using the program DISTLM (Anderson 2003).

Time trends in the relative frequency of states for traits found to be important in the dbRDA were visualized by plotting trait state centroids on a biplot of the year scores from the first two CA eigenvectors. Trait centroids were calculated as the average position along the CA axes for all species having a particular trait state. For traits composed of multiple categories, centroids for each of the constituent categories were plotted based on the average position of all species along the CA axes weighted by their score for that category. Only traits found to be significant in the dbRDA were plotted.

The utility of the dbRDA is that it explicitly considers the interannual changes in abundance of all species. However, part of the variance it seeks to explain is short-term interannual variability, of which observation error is a non-negligible component (the average coefficient of variation for the abundance estimates in the survey is 46%). Thus, we also examined how well the traits explained the main

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pattern of change over time in the species composition of the community, summarized by CA-I. We used a general linear model (GLM) with the species scores on CA-I as the univariate response variable (N = 52) and the species traits as explanatory variables.

Testing traits as indicators of hypothesized causal effects

A key assumption of the traits-based analysis is that the correlation between a trait and $\mathbf{D}_C$ reflects the effect of the forcing factor thought to be associated with that trait. We were able to test this assumption for two of the traits, biogeographic distribution and seal predation, as follows.

We expected biogeographic distribution to reflect thermal preferences and thus responses to changes in climate. We predicted that if the climate signal could be "filtered" out of the time series of species abundances, then the relationship between abundance and trait matrices should weaken for biogeographic distribution but not for the other traits. To test this prediction, new time series of abundance were constructed for each species based on the residuals from the regression of abundance, $\mathbf{C}_{ij}$, on the mean temperature in the cold intermediate layer (CIL: 30–150 m; –1 to 2 °C; Fig. 2*c*), the water layer that covers most of the bottom of the southern Gulf of St. Lawrence (Gilbert and Pettigrew 1997). The new abundance indices, controlled for the effect of changing climate, were again transformed to range over the interval [0,1]. The resulting $N \times Y$ residual abundance matrix, $\mathbf{R}_{\mathrm{CIL}}$, was used to calculate a dissimilarity matrix, $\mathbf{D}_{\mathbf{R}_{\mathrm{CIL}}}$, using the methods described previously. A Mantel test, based on ranks, was used to measure the respective $\mathbf{D}_{\mathbf{T}_t}$–$\mathbf{D}_{\mathbf{C}}$ and $\mathbf{D}_{\mathbf{T}_t}$–$\mathbf{D}_{\mathbf{R}_{\mathrm{CIL}}}$ correlations for each of the traits, t. Simultaneous bootstrapping (9999 iterations) of species in $\mathbf{C}$ and $\mathbf{R}_{\mathrm{CIL}}$, followed by recalculation of $\mathbf{D}_{\mathbf{C}}$ and $\mathbf{D}_{\mathbf{R}_{\mathrm{CIL}}}$ and then the Mantel correlations was used to estimate the probability that the $\mathbf{D}_{\mathbf{T}_t}$–$\mathbf{D}_{\mathbf{C}}$ correlations were weaker than the $\mathbf{D}_{\mathbf{T}_t}$–$\mathbf{D}_{\mathbf{R}_{\mathrm{CIL}}}$ correlations. This was done by counting the number of paired bootstrap samples in which the Mantel correlation based on the residual abundance trends was greater than or equal to that based on the original trends. Because there are often delays between climatic changes and population responses (e.g., Stenseth et al. 2004), the cold intermediate layer temperature series was filtered from abundance trends using lags of 0 to 5 years, and the analysis was repeated for each case.

A similar analysis was conducted by filtering out the effect of seal predation based on the regression of $\mathbf{C}_{ij}$ on grey seal abundance (Fig. 2*b*), lagging the seal index by 1 year, to produce the residual abundance matrix, $\mathbf{R}_{\mathrm{seal}}$. The remainder of the analysis proceeded as described above. In this case, however, a decrease in correlation strength was predicted for the seal predation trait, but not the other traits. A similar analysis could not be conducted for exploitation level because reliable time series of fishing mortality were lacking for many species. The same was true for diet and time series of prey abundance. Furthermore, because this analysis tests for a decline in the association between traits and community dynamics, only traits showing a significant association in the dbRDA were tested.

Finally, a null distribution was also generated using 10 000 iterations in which an $\mathbf{R}_{(\mathrm{random})}$ matrix was created using randomly generated environmental series. The expectation for the null model is that $\mathbf{D}_{\mathbf{T}_t}$–$\mathbf{D}_{\mathbf{R}_{(\mathrm{random})}}$ correlations would be stronger than $\mathbf{D}_{\mathbf{T}_t}$–$\mathbf{D}_{\mathbf{C}}$ correlations about 50% of the time.

Establishing absolute levels of removal by fisheries and seals

An important further step in evaluating the evidence for the hypothesized effects of fisheries and seals was to establish whether the absolute magnitude of fish removals by each of these two sources is sufficient to explain the changes observed in the community. Given the difficulties in quantitatively establishing the species composition of seal diets, we considered total annual removals of fish for the whole community. Annual grey seal consumption of fish prey, Q_j, was estimated using a mean annual individual consumption rate of 1.75 tonnes·seal^{-1} (calculated from Hammill and Stenson 2000) multiplied by the number of grey seals in the Gulf of St. Lawrence herd (Fig. 2*b*). Because there are seasonal movements between areas by seals in the Gulf herd (Harvey et al. 2008) and the neighboring Sable Island herd (Breed et al. 2006), estimated consumption of southern Gulf fish based on abundance of the Gulf herd alone is likely to be conservative given the much larger size of the Sable Island herd. Annual removals of fish by fisheries were summarized for landed (L_j) and discarded (D_j) catch by summing over species. Discarded catch was kept separate given that long-term survival of released fish is generally not well known (Davis 2002), though across all species and fisheries, we expect it to have been relatively low. The removals Q_j, L_j, and D_j were expressed as a percentage of catchability-adjusted fish standing stock biomass, B_j.

Top-down effects of fish predation

The final hypothesis, that the composition of the fish community is affected by changes in the amount of predation by piscivorous fishes, could not be evaluated using the traits-based approach. We could not identify a trait that unambiguously reflected susceptibility to predation by fishes. Thus, we used the more traditional approach of correlation, examining the relationship between relevant response and forcing factor variables.

We used the biomass of small-bodied fish (defined here as fish < 20 cm in length) as the response variable. This was estimated from catch rates (numbers) at length and length–weight data obtained for all fish species caught during the annual surveys. Catches were adjusted for catchability (see Appendix A, Table A1) and expanded from the area swept by a standard tow to the survey area.

The potential explanatory variable was an index of annual size-dependent piscivory (P_j) by the fish community (e.g., Duplisea 2005). P_j was calculated using an allometric relationship for total consumption combined with a correction for the proportion of fish in the diet of predators and size-dependent prey selectivities:

$$(3) \qquad P_j = 365 \sum_{i=1}^{N} \sum_{k=1}^{k_{\max}} A_{ijk}\,(0.01\, w_{ijk}^{0.75})\,\mathrm{PF}_{ik} \cdot C(<20)_{ik}$$

where A_{ijk} is the total catchability-adjusted abundance of fish predator species i in year j at length k, w_{ijk} is body weight at length k for species i in year j, PF_{ik} is the propor-

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Table 2. Summary of the principal questions addressed in this paper, including in each case the type of analysis used, the response and explanatory variables involved, how statistical significance was assessed, and how the results are presented.

Analysis	Response variable	Explanatory variable	Statistical testing	Results presented
How has the species composition changed over time?				
CA	$\mathbf{C}$	—	—	1. Plot of standardized species abundance time series sorted by the first CA eigenvector (CA-I) (Fig. 4) 2. Ordination of years with respect to the first two CA eigenvectors (Fig. 5)
Which (*i*) traits or (*ii*) suite of traits are (is) related to interspecies differences in interannual changes in abundance?				
(*i*) dbRDA (marginal tests)	$\mathbf{D}_{\mathbf{C}}$	$\mathbf{T}_t$	Permutation of the elements of $\mathbf{D}_{\mathbf{C}}$	1. Effect size (explained variance, $R^2_{\mathbf{V}\mid\mathbf{X}}$) and statistical significance (Table 3*a*) 2. Plot of trait values (Fig. 3) sorted in the same manner as the ordered species abundance time series (Fig. 4)
(*ii*) dbRDA (partial tests)	$\mathbf{D}_{\mathbf{C}}$	$\mathbf{T}_t$	Permutation of the multivariate residual matrix	1. Plot of trait centroids in ordination of years (Fig. 5) 2. Cumulative effect size ($R^2_{\mathbf{V}\mid\mathbf{X}}$) and individual statistical significance (Table 3*b*)
Which traits are related to the main pattern of change over time in species composition?				
GLM	CA-I species scores	$\mathbf{T}_t$	Parametric probability	Explained variance (R^2) and statistical significance presented in the text
How do the traits covary?				
Mantel correlation	$\mathbf{D}_{\mathbf{T}_t}$	—	Permutation of the elements of $\mathbf{D}_{\mathbf{T}_t}$	Table of pairwise Mantel correlation analysis results (Table 4) and plots of trait values (Fig. 3)
Does the correlation between a trait and the species dissimilarity matrix reflect the effect of the forcing factor thought to be associated with that trait?				
Mantel correlation	$\mathbf{D}_{\mathbf{C}}$, $\mathbf{D}_{\mathbf{R}_f}$, $\mathbf{D}_{\mathbf{T}_t}$	Time series of f, as covariates to produce $\mathbf{R}_f$	Simultaneous bootstrapping of $\mathbf{C}$ and $\mathbf{R}$	Mantel correlation coefficients and the approximate probability that the $\mathbf{D}_{\mathbf{T}_t}$–$\mathbf{D}_{\mathbf{C}}$ correlations were weaker than the $\mathbf{D}_{\mathbf{T}_t}$–$\mathbf{D}_{\mathbf{R}}$ correlations (Table 5)
Are the estimated absolute levels of removal consistent with the effects of fishing and seal predation?				
—	B_t, L_t, D_t, Q_t	—	—	Plot of removals by fisheries and seals as a percentage of estimated fish biomass (Fig. 6)
Is there evidence that the composition of the fish community is affected by changes in the amount of predation by piscivorous fishes?				
Correlation	P_t (eq. 3), B_t (l<20 cm)	—	Parametric probability[a]	Plot of both time series (Fig. 7) along with correlation and associated p value

Note: The following notation is used in this table and in the text: CA and dbRDA denote correspondence analysis and distance-based redundancy analysis, respectively; $\mathbf{C}$ is the matrix of standardized annual species catch rates; $\mathbf{R}_f$ is the matrix of standardized residual annual species catch rates following regressions on the time series of forcing factor f (either the cold intermediate layer temperature or grey seal abundance); $\mathbf{T}_t$ is the species matrix for trait t; $\mathbf{D}_{\mathbf{C}}$, $\mathbf{D}_{\mathbf{R}_f}$, and $\mathbf{D}_{\mathbf{T}_t}$ are, respectively, the species dissimilarity matrices based on the elements of $\mathbf{C}$, $\mathbf{R}_f$, and $\mathbf{T}_t$; B_t, L_t, D_t, and Q_t are, respectively, the total annual biomass, landings, discards, and grey seal consumption of marine fishes; GLM, general linear model.

[a]The probability of the data given the null hypothesis was based on degrees of freedom adjusted for autocorrelation.

tion of fish in the diet of species i at length k based on published accounts (Bowman et al. 2000; Hanson and Chouinard 2002; Savenkoff et al. 2004), and $C(<20)_{ik}$ is the fraction of the total fish consumption by species i of length k that is composed of fish prey less than 20 cm in length. The daily consumption rate (kg·individual^{-1}·day^{-1}) was estimated by the allometric relationship $0.01w^{0.75}$, based on the common exponent for metabolic rates (Brown et al. 2004) and a scaling parameter value used in multispecies virtual population analyses for North Sea cod (Magnússon 1995; ICES 1998). $C(<20)_{ik}$ was calculated using published linear relationships for minimum, average, and maximum prey sizes consumed as a function predator size (Scharf et al. 2000). For species without published relationships, we applied the relationships published for anatomically similar species: the cod relationships were used for other round fish, those for windowpane for the other flatfish, winter skate for the other skates, and spiny dogfish for black dogfish. Consumption was distributed among 1 cm prey size groups using one of two approaches: even distribution between the predicted minimum and maximum prey sizes or assuming the existence of a preferred prey size within the range of consumed prey. In the second approach, size preference was modeled using a normal distribution of consumption centered on the predicted mean prey size (with a standard deviation equal to one-sixth the difference between the maximum and minimum prey sizes). Both approaches produced similar temporal patterns in P_t, so results are pre-

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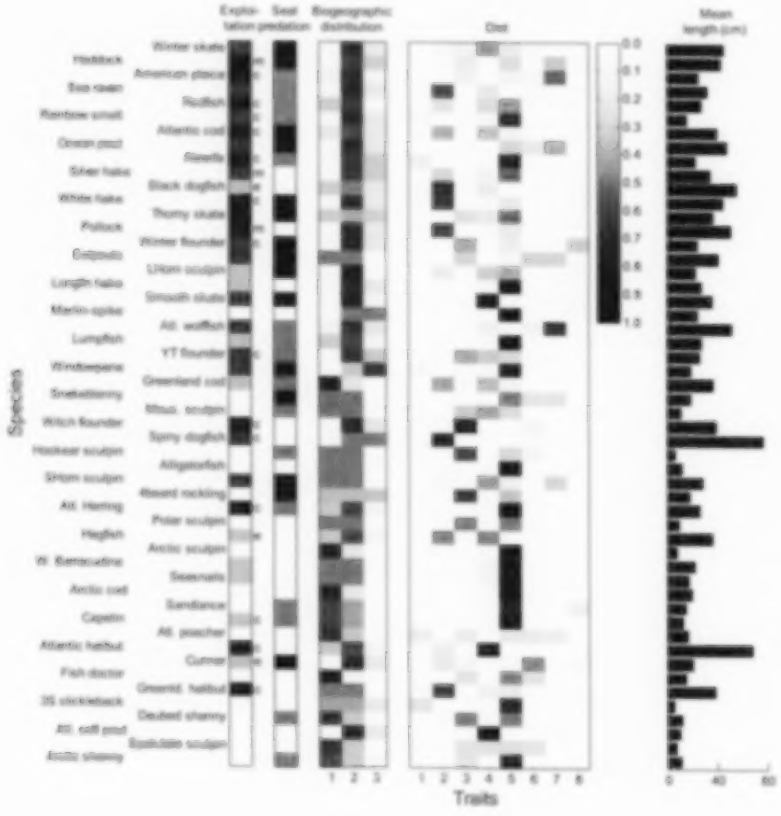
Fig. 3. Biological traits of the southern Gulf of St. Lawrence marine fish species. Values for exploitation level, seal predation, biogeographic distribution, and diet are represented by shading (according to the inset bar), whereas a histogram is used to display mean body length. Species were sorted by species scores on the first correspondence analysis eigenvector (CA-I). Letters to the right of exploitation level indicate whether species were captured in a directed commercial fishery (c), sought in a mixed-species commercial fishery (m) directed principally at another species, or captured in a new or experimental commercial fishery (e). For the biogeographic distribution trait, the categories are as follows: 1, Arctic–subarctic; 2, boreal; 3, temperate. For diet, the categories are as follows: 1, fish eggs and larvae; 2, adult fish and squid; 3, marine worms; 4, shrimps and crabs; 5, invertebrate plankton; 6, mollusks other than squid; 7, echinoderms; 8, other prey. The mean length of species measured in the survey was added to the plot to highlight changes that have occurred in the size structure of the community.



sented here only for the approach assuming preferred prey sizes.

A summary is provided of the analyses described throughout the Methods section, including their inputs and outputs (Table 2).

Results

The first CA eigenvector (CA-I; 27% explained variance) summarized major time trends in species abundance (correlation with time, $r = 0.94$). Sorting the species in **C** by their scores on CA-I and plotting their abundance highlights clear and dramatic changes in the composition of the southern Gulf marine fish community that have occurred since monitoring began in 1971 (Fig. 4). The magnitude of change was considerable for most species (see numbers provided in Fig. 4). Some species declined sharply in abundance in the late 1970s and early 1980s and have remained at low levels since then (e.g., American plaice, winter skate); others increased sharply in abundance in the late 1970s or late 1980s but suffered rapid population collapses in the 1990s (e.g., Atlantic cod, redfish); still others increased dramatically in abundance in the mid- or late 1990s (e.g., Greenland halibut, daubed shanny).

In marginal dbRDA tests, temporal variability in community composition was significantly related to the traits exploitation level, biogeographic distribution, and seal predation, but not to diet (Table 3*a*). These relationships are visually apparent when comparing Figs. 3 and 4, in which species are sorted in the same order. Over time, the community has shifted from one dominated by boreal species that are heavily fished (or incidentally captured in fisheries at a high rate) and commonly occur in grey seal diets to one dominated by species that are rarely caught in fisheries, occur at more northerly latitudes, and tend to be less affected by seal predation (Fig. 5).

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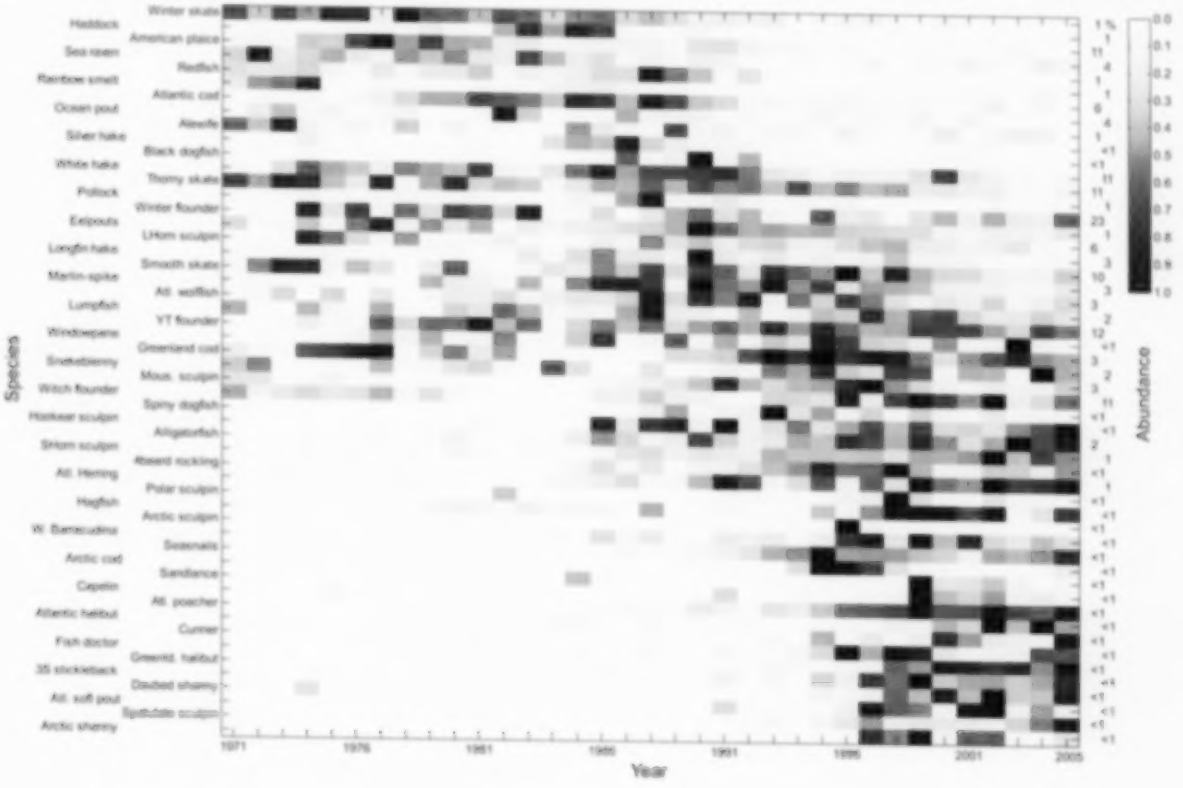
Fig. 4. Time series of abundance for 52 marine fish species (rows) in the southern Gulf of St. Lawrence, 1971–2005 (see Appendix A, Table A1 for scientific names). Abundance was transformed to range between 0 (lowest observed abundance, white) and 1 (highest observed, shaded black). Years with intermediate levels of abundance are shaded grey according to the bar on the right. The numbers immediately to the right of the plot are the minimum untransformed survey catch rate as a percentage of the maximum observed for each species, providing an indication of the magnitude of abundance change. As in Fig. 3, species are sorted by the species scores on CA-I to highlight the principal changes that have occurred in the community over time.



We found independent effects of the traits biogeographic distribution, exploitation level, and seal predation on the temporal variability in community composition using partial dbRDA (Table 3*b*). No significant relationships were found for diet in the partial analyses. Together, the three significant traits explained 25% of the multispecies abundance variation. However, intercorrelations among the traits (Table 4) limited somewhat the degree to which dissimilarity in abundance trends between species could be uniquely attributed to any one trait. By repeating the partial dbRDA with each trait in turn as the last-entered variable, we estimated that of the explained variation, 6% was uniquely attributable to biogeographic distribution, 5% to seal predation, and 4% to exploitation level, with the remaining 10% confounded among the three traits. In the univariate (GLM) analysis of long-term trends in species abundance, 55% of the variance in species scores on CA-I was explained by the three traits. The result for all three traits was statistically significant in marginal tests (in all cases, $p \leq 0.001$) and in conditional tests when entered as the last variable in the model (i.e., type III sums of squares; all $p < 0.05$).

The shift in community trait composition corresponds qualitatively with changes in the hypothesized forcing factors relevant to the traits. Much of the decline in abundance of the species most impacted by fisheries occurred during periods of high overall groundfish fishing mortality, particularly during the early 1990s (Fig. 2*a*). Likewise, the lack of recovery or continued decline of many of these species following large reductions in fishing effort in the early 1990s corresponds with dramatic increases in the abundance of grey seals in the ecosystem (Fig. 2*b*). Furthermore, the shift in the biogeographic origin of species coincides with changes in the mean bottom-water temperatures in the cold intermediate layer (Fig. 2*c*). The abundance of cooler-water species, particularly Arctic species, peaked during or shortly after the period of coldest water temperatures, declining somewhat subsequently as temperatures moderated (Figs. 3 and 4). Shorter-term temperature changes occurring during the 1970s do not appear to have led to comparable shifts in species composition (Fig. 5).

We found support for our assumption that the correlation between the trait biogeographic distribution and $\mathbf{D}_{\mathbf{C}}$ reflects an effect of changing water temperatures. When the cold intermediate layer temperature series was filtered from **C** to

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Table 3. Relationships between abundance trends and species traits in the southern Gulf of St. Lawrence marine fish community based on distance-based redundancy analysis (dbRDA).

Traits	Pseudo-F	p	$R^2_{Y\|X}$
(*a*) Marginal tests.			
Biogeographic distribution	3.28	**<0.0001**	17.0
Seal predation	4.37	**0.0007**	8.1
Exploitation level	7.94	**<0.0001**	13.7
Diet	1.25	0.1092	18.9
(*b*) Conditional test, forward selection.			
1. Biogeographic distribution	3.28	**<0.0001**	17.0[a]
2. Seal predation	2.80	**0.0041**	21.7[a]
3. Exploitation level	1.81	**0.0431**	24.7[a]
4. Diet	1.02	0.4397	32.0[a]

Note: $R^2_{Y|X}$ is the percentage of the multivariate species-abundance dynamics (matrix **Y**) explained by the trait(s) (matrix **X**) and is the multivariate equivalent of the regression coefficient of determination. Analyses yielding a probability value smaller than 5% are interpreted as being statistically significant and are indicated in bold.

[a]Cumulative.

Table 4. Results of pairwise Mantel correlation analyses of species–trait dissimilarity matrices (p, based on 9999 permutations).

	Trait			
Trait	2	3	4	5
1. Exploitation level	<0.001	0.056	0.003	<0.001
2. Biogeographic distribution	—	0.794	0.030	0.011
3. Diet	—	—	0.077	<0.001
4. Seal predation	—	—	—	0.065
5. Mean body length	—	—	—	—

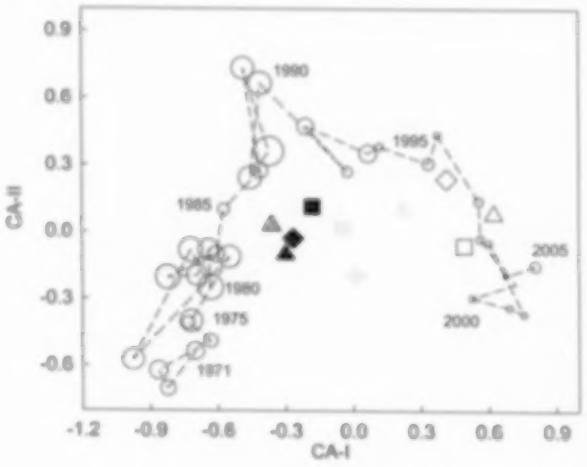
Note: Dissimilarities were calculated using the χ^2 distance for traits 2 and 3 and absolute arithmetic difference for the others.

produce $\mathbf{R}_{\text{(CH)}}$, the resulting $\mathbf{D_T}$–$\mathbf{D}_{\mathbf{R}_{\text{(CH)}}}$ Mantel correlations were weaker than the $\mathbf{D_T}$–$\mathbf{D_C}$ correlations (Table 5). This difference was observed for the temperature time series lagged by 0 to 3 years, though it was strongest and statistically significant (5% type I error level) for lags of 2 or 3 years. In contrast, yet also in line with prediction, there were no significant differences between $\mathbf{D_T}$–$\mathbf{D}_{\mathbf{R}_{\text{(CH)}}}$ and $\mathbf{D_T}$–$\mathbf{D_C}$ correlations for the exploitation level and seal predation traits for any time lag. Results for the null model analysis were consistent with our expectation of equally strong $\mathbf{D_T}$–$\mathbf{D}_{\mathbf{R}_{\text{(null)}}}$ and $\mathbf{D_T}$–$\mathbf{D_C}$ correlations (Table 5).

We also found support for the mechanism assumed to underlie the correlation between the seal predation trait and $\mathbf{D_C}$. When the grey seal abundance time series was filtered out of **C** to produce $\mathbf{R}_{\text{(seal)}}$, the $\mathbf{D_T}$–$\mathbf{D}_{\mathbf{R}_{\text{(s)}}}$ Mantel correlation was considerably weaker than the $\mathbf{D_T}$–$\mathbf{D_C}$ correlation for the seal predation trait (Table 5). Furthermore, as predicted, a statistically significant difference was not observed for the biogeographic distribution trait. In contrast with our predictions though, we observed a $\mathbf{D_T}$–$\mathbf{D}_{\mathbf{R}_{\text{(s)}}}$ correlation that was significantly weaker than its $\mathbf{D_T}$–$\mathbf{D_C}$ counterpart for the exploitation level trait, suggesting that the effects of exploitation and seal predation are somewhat confounded in our analyses. This confounding follows from a correlation between the traits believed to reflect susceptibility to fisheries and seal predation (Table 4) and a trade-off over time in the estimated removals by these two sources of mortality (Fig. 6). For the primary prey of seals, increased consumption by seals in the early to mid-1990s counteracted the sharp decline in fishery removals so that the total rate of removal due to fishing and seal predation remained roughly constant over the time series (Fig. 6*a*). This is less evident considering removals of all seal prey, though the contribution of seals to these removals generally increased over the series (Fig. 6*b*).

The estimated magnitude of combined removals by fishing and seals (~10% of total seal prey or ~25% primary prey biomass) supports these two sources of mortality as plausible causes of community change for seal prey species (Fig. 6). Likewise, for species captured by fisheries but not seals, an average of 37% (1971–1989) or 5% (1990–2005) of standing stock biomass was removed every year (figure not shown), levels of removal that make an effect of fishing plausible.

Fig. 5. Temporal trends in the species and trait composition of the southern Gulf of St. Lawrence marine fish community. The plot presents the ordination of years (circles) with respect to axes I and II of the correspondence analysis (CA) of fish species abundance in the southern Gulf (1971–2005). The axes explained 27.2% (λ_1 = 0.345) and 9.8% (λ_2 = 0.124) of the variability in abundance, respectively. The distance between years in the plot reflects their similarity in species composition. Trait centroids are plotted for the traits exploitation level (triangles: black, score =1 (high); dark grey, 0.67 (intermediate); light grey, 0.33 (low); open, 0 (no capture)), seal predation (diamonds: black, 1 (above median); grey, 0.5 (below median); open, 0 (no predation)), and biogeographic distribution (squares: black, temperate; grey, boreal; open, Arctic–subarctic). The survey-derived mean annual body length in the community is indicated by the size of the circle for each year (minimum 8 cm, maximum 24 cm). The proximity of trait centroids to a given year is an indication of the characteristics of the species dominating the community in that year (e.g., Arctic–subarctic species were a more important component of the community in the 2000s than in the 1970s).



The changes in species composition were also accompanied by a change in community size structure, with particularly sharp declines in mean length during the 1990s

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Table 5. Results of Mantel correlation analyses relating the dissimilarity matrices for three traits (biogeographic distribution, seal predation, and exploitation level) to the dissimilarity matrices based on (*i*) the original species–year matrix, and residual species–year matrices from (*ii*) the randomly generated environmental time series, (*iii*) the abundance of grey seals, and (*iv*) cold intermediate layer temperatures at various time lags.

	Species trait		
Filtered series	Biogeographic distribution	Seal predation	Exploitation
(*i*) None	0.16 (—)	0.12 (—)	0.15 (—)
(*ii*) Random series[a]	0.16 (0.474)	0.12 (0.501)	0.14 (0.546)
(*iii*) Seal abundance	0.11 (0.194)	0.01 (**0.011**)	0.03 (**0.027**)
(*iv*) Mean cold intermediate layer temperature[b]			
No lag	0.10 (0.088)	0.11 (0.511)	0.13 (0.390)
Lag 1 year	0.11 (0.099)	0.11 (0.532)	0.13 (0.363)
Lag 2 year	0.10 (**0.019**)	0.13 (0.626)	0.14 (0.446)
Lag 3 year	0.09 (**0.039**)	0.13 (0.660)	0.11 (0.332)
Lag 4 year	0.15 (0.325)	0.12 (0.319)	0.12 (0.235)
Lag 5 year	0.16 (0.452)	0.11 (0.300)	0.14 (0.445)

Note: Mantel correlation coefficients are presented along with, in parentheses, the approximate probability that the Mantel correlation with the residual species–year matrix is equal to or greater than that with the original matrix. Analyses yielding a probability smaller than 5% are indicated in bold.

[a]Mantel correlation values presented in (*ii*) are the mean for 10 000 simulations.

[b]Although time lags up to 10 years were initially analyzed, only those from 0–5 years are presented as all lags of 4 years or more produced similar results.

Fig. 6. Total annual fishery landings (grey), estimated fishery discards (open), and estimated grey seal consumption (black) as a percentage of fish standing stock biomass. The percentages in (*a*) are for only those species scored as 1 on the seal predation trait, based on the conservative assumption that 50% of total seal predation was concentrated on those species. Similarly, the percentages in (*b*) are for all seal fish prey (i.e., seal predation trait = [0.5,1]).

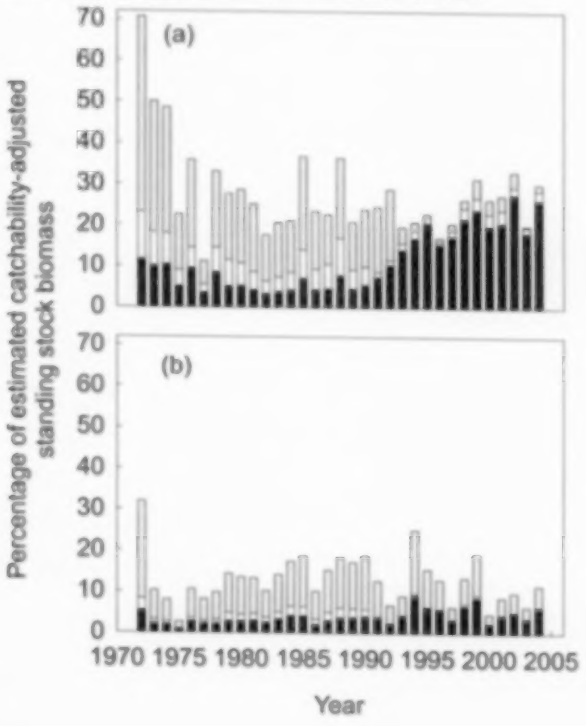
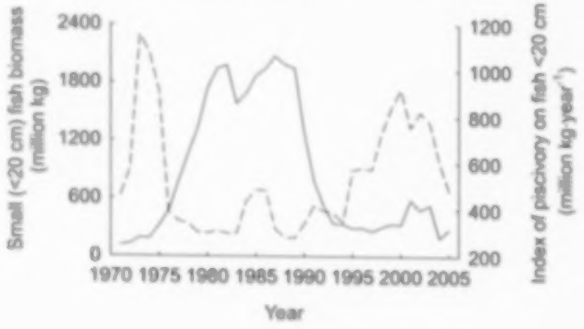


Fig. 7. Total annual biomass of fish <20 cm in length (broken line) and the index of potential predation by piscivorous fish on those small-bodied fish (solid line), 1971–2005. Both quantities are estimated using catchability-adjusted research survey catches and are presented as 3-year running averages.



(Fig. 5). These changes are characterized by both a decrease in abundance of many large-bodied species, particularly during the late 1980s, and an increase in smaller-bodied species during the 1990s (Figs. 3 and 4). We found opposite trends over time in total small fish biomass and in the index of predation on them by larger fish (Fig. 7; Spearman's correlation = –0.70, $p = 0.012$ based on degrees of freedom adjusted for autocorrelation following eqs. 1, 6, and 7 of Pyper and Peterman 1998). This result is consistent with a hypothesized effect of piscivory on community size structure. Size-dependent predation may not be the only cause of change, however, as many of the cooler-water species in the community are also smaller-bodied (Fig. 3). The correlation between the traits biogeographic distribution and body size (Table 4) suggests that changing water temperatures may have indirectly contributed to changes in

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size structure. However, only the patterns in size-dependent piscivory can explain the high biomass of small fishes during the early 1970s (Fig. 7).

Discussion

In this paper, we have adopted the view that the dynamics of an ecological community reflect changes over time in its environment, which selects for or against particular suites of functional traits and species tolerances (e.g., Keddy 1992). In our view, this traits-based approach has a number of advantages. First, the functional forms of species interactions and population responses to environmental forcing do not need to be specified (Shipley et al. 2006). This is advantageous, for example, when species population responses to external change are nonlinear (e.g., Mysterud et al. 2001; Stenseth et al. 2002). Second, by comparing the dynamics of numerous species with widely contrasting states across a suite of relevant traits, it may be possible to disentangle the responses to otherwise confounded multiple forcing factors. Third, a functional basis for expected responses of species to environmental change is the foundation of the approach. Functional models can also be used in traditional approaches, with the responses of individual species to forcing factors explicitly modeled; however, these traditional models become problematic as the number of species, and thus their interactions with each other and physical forcing factors, increase. However, despite these advantages, the traits-based approach remains essentially correlative. Thus, where possible, we attempted to strengthen our inference by examining the effect of filtering the underlying forcing factors from the time series of species abundance and by examining whether the effects of the hypothesized forcing factors (i.e., magnitude of fishery removals, seal consumption) were sufficient to have a substantial influence on community dynamics.

The traits-based approach also had a number of limitations in our case. First, only static (i.e., average) trait values could be used. This is a particular issue for the exploitation level trait because of changes in target species over time. However, because interspecific differences in exploitation rate generally exceeded intraspecific differences over time (results not shown), this trait was still a useful general measure of susceptibility to fishing. A second limitation was the use of semiquantitative traits. On one hand, the semiquantitative traits likely limited the variance in $\mathbf{C}$ explained in the dbRDA. On the other hand, some subjectivity in our choice of traits scoring may have influenced our results. Sensitivity to the scoring was, however, examined by repeating the dbRDA using traits that were scored more coarsely (e.g., reducing diet to four prey groups or seal predation to two levels). Results (not shown) were robust to these alternate scoring schemes. A third limitation was that it may not be possible to identify a trait that is unambiguously linked to a hypothesis of interest. In our study, this was the case for the hypothesized effect of predation by fishes. In the absence of a detailed food web of the southern Gulf community, susceptibility to predation by fishes could only be defined on the basis of body size. The problem is that body size in the community is also potentially affected by climate change (e.g., Blanchard et al. 2005), seal predation (e.g., Duplisea 2005), and the direct and indirect effects of fishing (e.g., Pope et al. 2006). The test of the hypothesized effect of predation by fishes was therefore necessarily ad hoc, though we again aimed to establish that both the magnitude and trend in predation were consistent with the inferred effects on the community. A final caveat with respect to our analysis is that it was focused largely on the dynamics of the adult and late juvenile stages (i.e., the size classes well sampled by the survey). This is particularly relevant to the hypothesis of bottom-up effects of changes in prey availability. Our analysis was based on the prey of adult fish, whereas bottom-up effects may be strongest at earlier life-history stages (e.g., Beaugrand et al. 2003).

Our analyses indicate that dramatic shifts in species composition of the fish community in the southern Gulf of St. Lawrence are related to the traits of these species. These traits, chosen to reflect susceptibility to particular forcing factors, allowed us to test four hypotheses for the causes of community change: direct fishing effects, climate change, changes in prey availability, and effects of a top predator, the grey seal. We found that three of these traits, the traits related to direct effects of fishing, climate change, and seal predation, could account for 25% of the interannual variability in the abundances of the 52 species in our analysis. The low percentage of variability explained by these traits may partly reflect observation error, an important component of the interannual variability in survey catch rates. Indeed we found that 55% of the variation in the species scores on CA-1, which summarized the main temporal trends in species composition, could be explained by these three traits.

Fishing is probably most often the factor implicated in marine fish community change (see the many examples in Hall 1999). This is not without cause given that mortality imposed by targeted fishing can be high, often exceeding that from natural sources (Stokes and Law 2000). Furthermore, incidentally captured (and discarded) sizes and species of fish often form a large proportion of total catches (e.g., Harrington et al. 2005). In the southern Gulf of St. Lawrence, every species that declined in abundance over the 35-year study period was susceptible to capture by fisheries. Much of this decline occurred during the late 1980s and early 1990s when fishing effort increased sharply. Declines were most pronounced for species with relatively high scores (0.67 and 1) on the exploitation level trait. These included both targeted species (e.g., Atlantic cod, redfish) and species that are incidentally caught and discarded (e.g., sea raven).

Exploited species that declined to low abundance in the 1990s have failed to recover despite very low levels of fishing for over a decade. This suggests a decline in the productivity of these populations (e.g., Shelton et al. 2006), possibly because of ecosystem change. The reduced productivity of Atlantic cod in the southern Gulf of St. Lawrence stems in large part from elevated natural mortality of adult cod (Sinclair 2001). Congruent trends between that mortality and grey seal abundance suggest the hypothesis that increasing predation by grey seals may contribute to the reduced productivity of this cod population (Chouinard et al. 2005). Our results are consistent with this hypothesis and suggest a broad impact of elevated seal predation on the fish community. Since the late 1980s, composition of the community has shifted towards species that are less impacted by grey seals.

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Among the large-bodied species susceptible to capture by fisheries, only those consumed at a low rate or not at all by seals have increased in abundance since the mid-1980s (e.g., witch flounder and Greenland and Atlantic halibuts).

Although our analyses did identify independent effects of the traits exploitation level and seal predation, these two sources of mortality were largely confounded, perhaps partly as a result of the use of static trait values. Among their primary prey in particular, increased consumption by grey seals replaced reduced fishery removals in the 1990s, providing an explanation for the lack of recovery of these fishes despite severely reduced fishing activity. Furthermore, the estimated magnitude of fish removals by fishermen and seals, though subject to the catchability adjustments used, supports these two sources of mortality as plausible causes of community change. The additional impact of other top predators such as harbour porpoise (*Phocoena phocoena*) is unknown given a lack of data on their diet and abundance.

Most fishes with infrequent capture in fisheries and low predation rates by seals increased dramatically in abundance in the 1990s. With few exceptions (e.g., Atlantic halibut), increasing species were small-bodied. Increased abundance implies reduced mortality from other sources, increased recruitment, and (or) increased immigration from other areas. One explanation for the recent increases in these small-bodied fishes is a release from predation following the depletion of large fish by fisheries (e.g., Blanchard et al. 2005; Frank et al. 2005). This explanation is supported by the inverse relationship observed between the estimated biomass of small fish and the consumption of these fish by large fish. The relative ranges of estimated small fish biomass (300–2300 million kg) and their consumption by large fish (200–1100 million kg·year^{-1}), though contingent on the assumptions made in estimating them, further make this mechanism plausible.

Effects of fishing and predation by seals and large piscivorous fish on the composition of this fish community suggest strong consumer control of fish production. In this case, these top-down effects all appear to reflect direct or indirect impacts of harvesting. Reduced predation by overexploited large piscivorous fish in the 1990s is likely an indirect effect of fishing. Likewise, increased predation by grey seals in the 1990s may be an indirect effect of reduced harvests and population control activities since the 1970s (Zwanenburg and Bowen 1990). Indirect harvesting impacts have also been proposed to explain reduced recruitment success of southern Gulf cod as stocks of Atlantic herring and Atlantic mackerel (*Scomber scombrus*), potential predators of cod eggs and larvae, rebuilt in the 1980s following reductions in harvesting (Swain and Sinclair 2000).

Our analyses also identified a significant effect of climate on the composition of the southern Gulf of St. Lawrence fish community. This effect was largely independent of those of fishing and seal predation and was linked to variation in bottom water temperatures. Community composition shifted towards species with more northerly biogeographic distributions following the cooling of the cold intermediate layer in the late 1980s and early 1990s. This mirrors a similar shift in zooplankton community composition in the Labrador Sea following cooling conditions (Beaugrand et al. 2002). The southern Gulf fish community appears to have responded to changing thermal conditions within 3 years. The range of possible time lags associated with the community changes suggests that the mechanisms underlying them may have varied between species. These mechanisms include climate-driven shifts in distribution (e.g., Beaugrand et al. 2002; Perry et al. 2005), environmental influences on demographic rates (e.g., Attrill and Power 2002), and a combination of the two (e.g., McGowan et al. 1998). The sudden appearance of species such as polar sculpin and Arctic sculpin, coincident with the occurence of unusually cold water temperatures in the mid-1990s and their rapid disappearance as temperatures warmed, is consistent with a distributional shift. The same is true of the relationship observed between the broader-scale distribution of capelin in the Gulf and the spatial extent of cold water (Grégoire et al. 2004). For other species such as Atlantic and Greenland halibuts, increases in the abundance of juvenile individuals in the southern Gulf (Benoît et al. 2003) are consistent with possible climate-mediated increases in recruitment.

We found no evidence, however, for bottom-up effects of environmental change acting through the prey of adult fish. (The relatively high proportion of variation explained by diet in the dbRDA is a reflection of the large number of categories used for this trait.) However, we can not rule out the existence of prey-mediated increases in certain individual species or bottom-up effects of changes in preferred prey of juvenile fish. Furthermore, an assumption underlying our analyses, that changes in prey availability should have similar effects on species with similar diets, may be invalid given expected effects of interspecific competition (e.g., Duplisea and Blanchard 2005). We can only say that the other effects considered — fishing, seal predation, and climate change — explain more of the changes observed in the survey of the southern Gulf fish community.

Our analyses point to direct impacts on species productivity stemming from changes in environmental conditions and mortality due to key predators (fish, seals, and ultimately humans). Interactions between these causal factors, though not addressed in our analysis, have the potential to add complexity to community dynamics. For example, differential species responses to climate change may affect existing local trophic relationships (Edwards and Richardson 2004) and the population dynamics of those interacting species (Davis et al. 1998; Petchey et al. 1999). Also, the consequences of fishing are likely to depend on how species resilience and productivity are affected by climate change (e.g., Mora et al. 2007; Hsieh et al. 2006; Rose 2004) and top predators (e.g., Trzcinski et al. 2006). The potential for such interactive effects combined with our observed additive effects of multiple causes of community change underscore the need to adopt an ecosystem-based approach to management (Link 2002; Pikitch et al. 2004). However, given the suddenness of many of the changes observed here and elsewhere (Mullon et al. 2005), accelerating rates of climate change (Stainforth et al. 2005), and limited abilities to isolate direct, let alone indirect, effects of various drivers of community change, the effectiveness of both contemporary single-species and ecosystem-based fishery management is at risk of being "too little, too late". In the face of such a situation, we agree with others (Botsford et al. 1997) that greater precaution in management is prescribed.

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Appendix A

Table A1 appears on the following pages.

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Table A1. Common and scientific names of species included in the southern Gulf of St. Lawrence fish community analysis.

Class–Order–Family	Genus–species	Common name	*Q*
Myxini			
Myxiniformes			
Myxinidae	*Myxine glutinosa*	Atlantic hagfish	3
Chondrichthyes			
Rajiformes			
Rajidae	*Amblyraja radiata*	Thorny skate	5
	Leucoraja ocellata	Winter skate	5
	Malacoraja senta	Smooth skate	5
Squaliformes			
Etmopteridae	*Centroscyllium fabricii*	Black dogfish	4
Squalidae	*Squalus acanthias*	Spiny dogfish	4
Actinopterygii			
Aulopiformes			
Paralepididae	*Arctozenus risso*	White barracudina	3
Clupeiformes			
Clupidae	*Alosa pseudoharengus*	Alewife	6
	Clupea harengus	Atlantic herring	6
Gadiformes			
Gadidae	*Boreogadus saida*	Arctic cod	4
	Gadus morhua	Atlantic cod	1
	Gadus ogac	Greenland cod	3
	Melanogrammus aeglefinus	Haddock	2
	Pollachius virens	Pollock	4
Macrouridae	*Nezumia bairdii*	Marlin-spike	3
Merlucciidae	*Merluccius bilinearis*	Silver hake	4
Phycidae	*Enchelyopus cimbrius*	Fourbeard rockling	3
	Phycis chesteri	Longfin hake	4
	Urophycis tenuis	White hake	3
Gasterosteiformes			
Gasterosteidae	*Gasterosteus aculeatus*	Threespine stickleback	4
Salmoniformes			
Osmeridae	*Mallotus villosus*	Capelin	6
	Osmerus mordax mordax	Rainbow smelt	6
Perciformes			
Ammodytidae	*Ammodytes dubius*	Northern sand lance	7
Anarhichadidae	*Anarhichas lupus*	Atlantic wolffish	3
Labridae	*Tautogolabrus adspersus*	Cunner	3
Stichaeidae	*Leptoclinus maculatus*	Daubed shanny	3
	Lumpenus lampretaeformis	Snakeblenny	3
	Stichaeus punctatus	Arctic shanny	3
Zoarcidae	*Gymnelis viridis*	Fish doctor	3
	Lycodes sp.	Eelpouts	3
	Melanostigma atlanticum	Atlantic soft pout	3
	Zoarces americanus	Ocean pout	3
Pleuronectiformes			
Pleuronectidae	*Glyptocephalus cynoglossus*	Witch flounder	5
	Hippoglossoides platessoides	American plaice	5
	Hippoglossus hippoglossus	Atlantic halibut	5
	Limanda ferruginea	Yellowtail flounder	5
	Pseudopleuronectes americanus	Winter flounder	5
	Reinhardtius hippoglossoides	Greenland halibut	5
Scophthalmidae	*Scophthalmus aquosus*	Windowpane	5
Scorpaeniformes			
Agonidae	*Aspidophoroides monopterygius*, *Ulcina olrikii*	Alligatorfishes	3
	Leptagonus decagonus	Atlantic poacher	3

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Table A1 (*concluded*).

Class–Order–Family	Genus–species	Common name	*Q*
Cottidae	*Artediellus* sp.	Hookear sculpins	3
	Icelus spatula	Spatulate sculpin	3
	Myoxocephalus octodecemspinosus	Longhorn sculpin	3
	Myoxocephalus scorpioides	Arctic sculpin	3
	Myoxocephalus scorpius	Shorthorn sculpin	3
	Triglops murrayi	Moustache sculpin	3
Cyclopteridae	*Cyclopterus lumpus*	Lumpfish	3
Liparidae	*Liparis* sp.	Seasnails	3
Hemitripteridae	*Hemitripterus americanus*	Sea raven	3
Psychrolutidae	*Cottunculus microps*	Polar sculpin	3
Scorpaenidae	*Sebastes* sp.	Redfish	4

Note: *Q* refers to the taxa- and length-dependent catchability corrections applied to the survey catches for each species to obtain estimates of the absolute abundance. Five of these models, based on species or species groups, were presented in Harley and Myers (2001): (1) Atlantic cod, (2) haddock, (3) demersal gadoids–roundfish, (4) pelagic gadoids, and (5) flatfish. Using stock assessment data for southern Gulf Atlantic herring, we fitted a similar model to apply to the (6) pelagic fishes (unpublished analyses). Finally, a length-aggregated correction for northern sand lance (7) was obtained from Sparholt (1990).

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Is ecological segregation in a pair of sympatric coregonines supported by divergent feeding efficiencies?

Jan Ohlberger, Thomas Mehner, Georg Staaks, and Franz Hölker

Abstract: Some of the sympatric species pairs commonly described in temperate freshwater fishes provide evidence for ecological specialization driven by competition for food resources as a potential prerequisite of subsequent sympatric speciation. In the postglacial Lake Stechlin (Germany), two sympatric coregonines coexist, common vendace (*Coregonus albula*) and endemic dwarf-sized Fontane cisco (*Coregonus fontanae*). The species segregate vertically along the light intensity and prey density gradients of their pelagic environment. Accordingly, we hypothesized that the species might show differences in their foraging efficiency associated with these environmental gradients. We investigated the feeding behaviour by measuring the functional response of both species to *Daphnia magna* at various prey densities (0.25–8 individuals·L^{-1}) and light intensities (0.005–5 lx) at a deep blue light spectrum to simulate their natural habitat. Decreasing light intensity and prey density significantly depressed consumption rates in both species. Overall, we observed only weak differences in feeding behaviour, which indicates that the species are functionally similar, coexisting planktivores.

Résumé : Quelques-unes des paires d'espèces sympatriques couramment décrites chez les poissons d'eau douce des régions tempérées montrent des signes de spécialisation écologique reliée à la compétition pour les ressources alimentaires, ce qui est potentiellement une condition préalable pour une spéciation sympatrique subséquente. Dans le lac Stechlin (Allemagne), un lac post-glaciaire, coexistent deux corégoninés sympatriques, le corégone blanc (*Coregonus albula*) et le corégone de Fontane (*Coregonus fontanae*), une espèce endémique naine. Il y a ségrégation verticale des espèces le long des gradients d'intensité lumineuse et de densité de proies dans leur milieu pélagique. C'est pourquoi, nous formulons l'hypothèse selon laquelle les espèces devraient montrer des différences d'efficacité de recherche de nourriture en relation avec ces gradients environnementaux. Nous avons étudié leur comportement alimentaire en mesurant la réponse fonctionnelle des deux espèces à *Daphnia magna* à diverses densités de proies (0,25–8 individuals·L^{-1}) et sous différentes intensités lumineuses (0,005–5 lx) dans un spectre de lumière bleu foncé afin de simuler leur habitat naturel. Une intensité lumineuse et une densité de proies décroissantes réduisent les taux de consommation chez les deux espèces. Globalement, nous n'observons que de légères différences de comportement alimentaire, ce qui indique que les espèces sont des planctonophages qui coexistent tout en affichant un fonctionnement similaire.

[Traduit par la Rédaction]

Introduction

Sympatric species pairs have been most commonly described in several families of teleost fishes in temperate postglacial lakes, basically in sticklebacks, smelts, and salmonids, including the coregonines (Taylor 1999). Many of these species pairs show clear ecological segregation (Schluter 1996, 2000), wherefore much of the recent research in this field has focused on the role of ecological specialization in driving and maintaining divergence in sympatry. There is ample evidence that the process is often driven by competition for food resources (Robinson and Wilson 1994; Smith and Skúlason 1996), and evidence for the process of sympatric speciation has been provided for some of these species pairs (e.g., Gislason et al. 1999; Knudsen et al. 2006).

Sympatrically living species are common among coregonines (whitefishes and ciscoes; see Hudson et al. 2007). Many of them exhibit profound differences in feeding strategies and habitat preferences (e.g., Lu and Bernatchez 1999; Amundsen et al. 2004). However, species pairs that do not display a divergence in diet or habitat also co-occur, for instance in the North American Lake Superior (Smith and Todd 1984; Turgeon et al. 1999). In these cases, alternative segregating strategies that contribute to the coexistence of the species have not been described so far. Since many environmental factors such as temperature, turbidity, and especially the light regime influence the feeding behaviour of

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planktivorous fish (Matthews 1998; Wootton 1998), diverse feeding efficiencies of sympatric planktivorous species might be expected if the species segregate along environmental gradients.

It is generally assumed that fish vision is well adapted to the light characteristics of the environment (Lythgoe 1979), and spectral adaptations have been described in many deepwater marine fishes (Lythgoe 1988). Planktivorous coregonines, like most other fish species (Guthrie and Muntz 1993), are particulate feeders depending on vision to catch their prey (Janssen 1978; Dabrowski and Jewson 1984), and light intensity is considered to be an important factor controlling their feeding activity (Aksnes and Giske 1993). Fish feeding under sufficient light conditions benefit from better visibility of prey as well as higher reactive volume (Townsend and Risebrow 1982; Ryer et al. 2002; Pekcan-Hekim and Horppila 2007). However, most of our understanding of fish feeding ecology is based on studies conducted under moderate to high light levels, and foraging models for planktivores assume negligible feeding rates at extremely low light intensity (Giske and Salvanes 1995; Hölker and Breckling 2001). Moreover, most studies employ a light spectrum commonly used for room illumination, although the spectrum of the light changes considerably with increasing water depth towards predominantly blue wavelengths (Jerlov 1976; Gerking 1994). It is known that the spectral composition influences the consumption rate of zooplanktivorous fish, because reaction distance is reduced as the wavelength of light decreases from red to blue (Dabrowski and Jewson 1984; Henderson and Northcote 1985).

In the deep and oligotrophic Lake Stechlin (Germany), vendace (*Coregonus albula*) is the dominant pelagic fish species living in sympatry with the endemic dwarf-sized Fontanae cisco (*Coregonus fontanae*; Schulz and Freyhof 2003). The species are easily distinguished by spawning time, with vendace spawning in winter and Fontanae cisco spawning from spring to summer. The species have been described distinct by morphological characteristics (Schulz and Freyhof 2003), and it has been suggested from mtDNA and microsatellite analyses that this species pair evolved via sympatric speciation (Schulz et al. 2006). Both species are planktivores inhabiting the pelagic habitat where they perform diel vertical migrations by ascending from their deepwater daytime habitat into shallower water layers during nighttime (Helland et al. 2007; Mehner et al. 2007). No species-specific difference in diet composition has been observed (Schulz et al. 2003; Helland et al. 2008), and this contrasts the commonly observed divergence along the limnetic–benthic axes found in many other sympatric species in temperate freshwater systems (Lu and Bernatchez 1999; McKinnon and Rundle 2002; Knudsen et al. 2006). Habitat segregation between the Lake Stechlin coregonines has only been observed with respect to vertical distribution within the pelagic habitat (Helland et al. 2007) with Fontane cisco occurring on average in deeper waters than vendace. Zooplankton density, besides light intensity and temperature, also declines continuously with depth in Lake Stechlin (Mehner et al. 2005). Moreover, owing to the weak ecological segregation, high exploitative competition between the species can be assumed. Hence, segregating strategies for reducing interspecific competition were expected according to the competition exclusion principle (see Pianka 2000).

Accordingly, we hypothesized that the species would show a divergence in their efficiency to consume zooplankton. Such a divergence would suggest heritable trait differences, since the fish were hatched and raised under the same laboratory conditions. We investigated the influence of light intensity and prey density on the functional response curves of both species. We hypothesized that Fontane cisco is more efficient at those low light levels and low prey densities, which are typical for the deepwater areas, whereas vendace should be superior at the brighter light and higher prey densities found in lower water depths, where this species is commonly found.

Materials and methods

Study species

Fontane cisco and vendace were caught by gillnetting (15 mm mesh size) during their respective spawning times in Lake Stechlin (53°10′N, 13°02′E), Germany. Ripe adults were striped and propagated artificially, and both species were raised under identical laboratory conditions. Larvae were hatched in glass aquaria and subsequently raised in circular basins (80 L). They were fed with brine shrimp, rotifers, and commercially available dry food for fish larvae. Juveniles were maintained in 500 L basins and fed with salmon dry food and *Daphnia magna* to habituate to this prey type. The fish were held at the experimental temperature of 8 °C for at least 2 weeks before experiments started. The prey species *Daphnia magna* was held in circular basins fed with *Desmodesmus subspicatus* algae.

Experimental design

Functional response tests were carried out in six 110 L cubic glass aquaria (48 cm × 48 cm × 48 cm) that were placed in a climatic chamber. The aquaria were filled with 80 L aerated fresh water up to a fill level of 35 cm. The climatic chamber was darkened with light-impermeable curtains inside and outside the entrance. Another row of dark curtains was installed as blinds in front of the aquaria with small peepholes for observation of the feeding activity. Small plastic reservoirs above each of the aquaria served as holding units for *Daphnia* for the feeding experiments. The outlets of these reservoirs were equipped with a valve that opened a tube for the introduction of the *Daphnia* into the aquaria approximately 10 cm below the water surface, which resulted in a homogeneous distribution of the *Daphnia* throughout the entire arena within a short time period. The valves were connected via a stick to a hand gear outside the blinds to introduce the prey without disturbing the fish. Luminaries equipped with 60 W halogen lamps were installed above each of the aquaria. The lamps were connected to a six-channel digital–analogue dimmer (Eurolight LD6230, Behringer, Germany) to allow fine-tuning of the light intensity at very low levels. The intensity of the light was set on a percent scale according to an exponential transmission characteristic of the dimmer. The color temperature of the flashlights (2800 kelvin (K)) was converted to approximately 20 000 K using tungsten conversion filters (E-Colour+, Rosco, UK). It was adjusted to this value by

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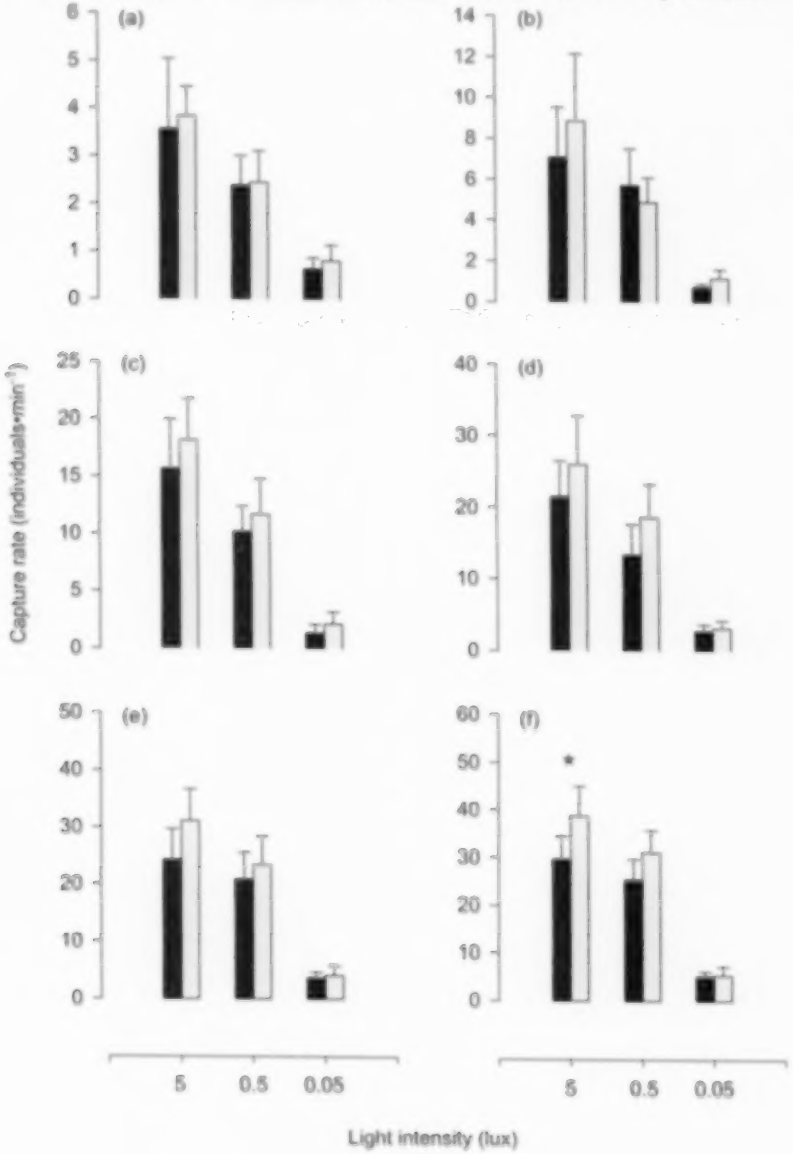
Fig. 1. Capture rates (mean and standard error) of Fontane cisco (*Coregonus fontanae*, black bars) and vendace (*Coregonus albula*, grey bars) at 5, 0.5, and 0.05 lx for all prey densities (individuals·L^{-1}): (*a*) 0.25; (*b*) 0.5; (*c*) 1; (*d*) 2; (*e*) 4; (*f*) 8. Differences between light intensities were significant between 0.5 and 0.05 lx at all prey densities in both species, but only partly significant between 5 and 0.5 lx. The only significant difference between species was found at 8 individuals·L^{-1} at 5 lx, as indicated by an asterisk.



placing two conversion filters (Double and Quarter C.T. Blue) on top of each other in front of the lamps behind a framed glass. Color temperature varied from 17,000 to 23,000 K depending on the light intensity used for the respective experiment. The conversion was done to simulate the blue spectrum of the natural habitat, since these fish usually feed in depths of more than 15 m during crepuscular to dark light periods and more than 35 m during daytime according to average year-round population depths (Helland et al. 2007; Mehner et al. 2007). Color temperature was measured with a color analyzer (TF5, Thoma, Germany). Light intensity was measured at mean depth in the aquaria using a light detector (SUD033/Y/W) connected to a research radiometer (IL 1700, International Light Technologies, Massachusetts, USA). The light detector had a measurement range of 400–700 nm and a dynamic range of 8×10^{-4} to 8×10^{5} lx.

Infrared lamps (880 nm, 60 W, WRL-I/LED06, Videor Technical, Germany) were installed above each of the aquaria to allow the observation of feeding activity at very low

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Table 1. *P* values of the analysis of variance (ANOVA) Tukey's test for differences between light intensities at all prey densities.

	Coregonus albula		*Coregonus fontanae*	
Prey·L^{-1}	5 vs. 0.5 lx	0.5 vs. 0.05 lx	5 vs. 0.5 lx	0.5 vs. 0.05 lx
0.25	0.056	0.022	0.141	0.013
0.5	0.022	0.036	0.842	0.004
1	0.014	<0.001	0.026	<0.001
2	0.110	<0.001	0.063	0.008
4	0.044	<0.001	0.751	<0.001
8	0.071	<0.001	0.645	<0.001

Note: In both species, differences between 5 and 0.5 lx were partly significant, whereas differences between 0.5 and 0.05 lx were entirely significant.

Table 2. *P* values of the analysis of variance (ANOVA) Tukey's test for differences between species at all prey densities and light intensities.

Prey·L^{-1}	5 lx	0.5 lx	0.05 lx
0.25	0.990	0.999	0.999
0.5	0.640	0.978	0.999
1	0.696	0.949	0.997
2	0.570	0.409	0.999
4	0.092	0.908	0.999
8	0.016	0.404	0.999

Note: The only significant difference in consumption rate was found at the highest light intensity (5 lx) and prey density (8 individuals·L^{-1}) combination.

light intensities. This was done using night-vision glasses (D-2MV PRO, Hupra, Germany), which allowed the observation of the fish as well as the *Daphnia* during the experiments. Hence, it was possible to track an individual fish and its consumption of *Daphnia* through the peepholes of the aquaria without disturbing it.

Experimental protocol

Functional response experiments were performed at a water temperature of 8 °C at four different light intensities (0.005 ± 0.001, 0.05 ± 0.005, 0.5 ± 0.02, and 5 ± 0.01 lx). Six prey densities of 0.25, 0.5, 1, 2, 4, and 8 *Daphnia*·L^{-1} were used at each of the four light intensities. These prey densities were the mean densities over a feeding trail where each fish was allowed to eat six *Daphnia*. Accordingly, the total number of *Daphnia* introduced into the 80 L aquaria was 23, 43, 83, 163, 323, and 643 individuals for the six densities, respectively. Since the experiments were terminated after six *Daphnia* were captured, prey depletion was 26.1%, 14.0%, 7.2%, 3.7%, 1.9%, and 0.9% for the increasing prey densities, respectively. We therefore assume that the modest ratios of prey depletion did not substantially bias the results.

One fish was placed in each of the six aquaria, which thereby served as replicates for the specific light intensity and prey density combinations. The subsequent acclimation to experimental conditions was allowed overnight at the light intensity of the upcoming experiment. Individuals were randomly selected from a 500 L tank holding all potential experimental fish. Specimens used for this study were all juveniles and measured 6–11 cm (mean ± standard deviation, SD: 8.3 ± 1.3 cm) and 6–13 cm (mean ± SD: 9.7 ± 1.7 cm) total length for Fontane cisco and vendace, respectively. On the next day, prior to the experiments, *Daphnia* of 1.5–3 mm in size were sieved out of their holding tanks, counted, and poured into the small plastic reservoirs above the aquaria. After another 3 h, trails started with the introduction of the prey into one aquarium at a time. The time from the beginning of the feeding activity until the fish had eaten six *Daphnia* was measured using a stopwatch with an intermediate memory function. Typically, at the beginning of an experiment the fish stayed near the bottom. After perceiving the first *Daphnia*, the fish started to swim more actively, thereby searching for prey. At that time the *Daphnia* were already quite evenly distributed throughout the arena. The fish usually used the entire arena to forage continuously. After all six trails were counted, the fish were removed from the aquaria. Subsequently, the water was sieved to remove the remaining *Daphnia* and to prepare for the next experiment.

Data analysis

Three types of functional responses are distinguished: (*i*) the linear type I response with consumption rate increasing linearly with prey density until a satiation value is reached, with $N = aP$, where N is the consumption rate (individuals·L^{-1}), P is prey density (individuals·L^{-1}), and a is the slope of the consumption rate; (*ii*) the cyrtoid type II response with consumption rate increasing with prey density at a decreasing rate until satiation is reached, with $N = \beta_1 P(\beta_2 + P)^{-1}$, where β_1 is the maximum consumption rate, and β_2 is the prey density at which the consumption rate reaches half of its maximum (Holling 1959; Arditi and Ginzburg 1989); and (*iii*) the sigmoid type III response with consumption rate accelerating first with increasing prey density and thereafter decelerating towards satiation, with $N = \beta_1 P^2(\beta_2 + P^2)^{-1}$. We used linear and nonlinear regression analyses to fit these functional response curves.

A method based on information criterion was used to determine the regression model for the functional response that best described the experimental data. We used the Schwarz–Bayesian information criterion (S–BIC, Schwarz 1978). It is a restrictive criterion on increasing parameter numbers: $\text{S–BIC} = k\ln(n) + n\ln(\text{RSS}\cdot n^{-1})$, where k is the given number of parameters, n is the number of observations, and RSS is the residual sum of squares. The model associated with smallest value of S–BIC is the most appropriate.

Statistics

Functional response data were compared for significant differences using the following tests based on a significance level of $P < 0.05$. Analysis of variance (ANOVA, Tukey's test) was used to compare the mean values between species and light intensities at a particular prey density. To evaluate the influence of fish length, an analysis of covariance (ANCOVA) was performed. Further, we linearized the functional response data by taking the $\log_{10}$ of

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Table 3. Statistical results and selection criterion for the best-fit models of the functional response according to types I, II, and III curves for both species at all three light intensities.

	Coregonus albula			*Coregonus fontanae*		
Model	Type I	Type II	Type III	Type I	Type II	Type III
5 lx						
k	1	2	2	1	2	2
n	33	33	33	33	33	33
df	32	31	31	32	31	31
RSS	3369.92	786.78	1000.82	3226.95	1062.13	1097.19
S–BIC	156.16	111.65	119.59	154.73	121.55	122.63
0.5 lx						
k	1	2	2	1	2	2
n	31	31	31	33	33	33
df	30	29	29	32	31	31
RSS	1732.65	646.66	728.51	1333.18	347.62	432.05
S–BIC	128.16	101.04	104.74	125.56	84.70	91.87
0.05 lx						
k	1	2	2	1	2	2
n	30	30	30	29	29	29
df	29	28	28	28	27	27
RSS	70.20	35.85	44.53	37.67	27.10	32.38
S–BIC	28.90	12.15	18.65	10.95	4.76	9.93

Note: The model associated with the lowest values in S–BIC was selected as the most appropriate. Abbreviations are as follows: *k*, number of parameters used; *n*, total number; df, degrees of freedom; RSS, residual sum of squares; S–BIC, Schwarz–Bayesian information criterion.

prey density and compared the capture rate with prey density relationship between the species by ANCOVA. ANOVA and ANCOVA tests were performed using SPSS 14.0 (SPSS Inc., Chicago, Illinois). The estimated mean values for β_1 and β_2 were tested for significance using a *t* test.

Results

Capture rates in both species generally increased with prey density. Mean capture rates for both species at all prey densities and light intensities are shown (Fig. 1). No feeding activity was observed at 0.005 lx. In both species, differences between light intensities were all significant when 0.5 and 0.05 lx were compared, but only partly significant when 5 and 0.5 lx were compared (ANOVA, Tukey's test, Table 1). Significant differences between the species were found at the highest light intensity and prey density combination (5 lx and 8 individuals·L^{-1}). All other 17 interspecies comparisons were not significant. The differences between the species generally tend to increase with increasing light intensity and prey density (ANOVA, Tukey's test, Table 2). Fish size, measured as total body length, did not significantly affect capture rates (ANCOVA, $P > 0.1$ at all prey densities).

According to the S–BIC, the functional response type II gave the best fits to the data sets at all light intensities (5, 0.5, and 0.05 lx) in both species. However, the type III function gave comparably good fits in some cases (Table 3). The increase in capture rate with prey density at all light intensities is shown (Fig. 2). Capture success was depressed with decreasing light intensity in both species. After log-transforming prey density, ANCOVA showed a significant difference between the species at 5 lx ($P = 0.003$), but no significant difference at 0.5 lx ($P = 0.051$) and 0.05 lx ($P = 0.129$). The estimated parameters of the functional response type II fits are presented (Fig. 3, Table 4). According to the *t* test, maximum feeding rates were significantly higher in vendace compared with Fontane cisco at 5 lx (df = 10, $P = 2.839$), but there was no significant difference between species at 0.5 lx (df = 10, $P = 1.568$) and 0.05 lx (df = 10, $P = 0.563$). Differences between light intensities were not significant between 5 and 0.5 lx, but were significant between 0.5 and 0.05 lx in both species. Prey densities of half maximum feeding rate were not significantly different between species at all light intensities (5 lx: df = 10, $P = 0.699$; 0.5 lx: df = 10, $P = 0.284$; 0.05 lx: df = 8, $P = 1.151$).

Discussion

The consumption rate of Fontane cisco and vendace feeding on *Daphnia magna* increased with increasing prey density at a decreasing rate until satiation was reached. It thus followed a type II functional response. This type of response has been reported for other coregonines, for instance bloater (*Coregonus hoyi*; Miller et al. 1992), and was found in a recent study on a Swedish vendace population (U. Beier, Swedish Board of Fisheries, Drottningholm, Sweden, unpublished data). Some studies have reported a linear type I or a sigmoid type III functional response for planktivores (e.g., Winkler and Orellana 1992; Ryer et al. 2002), and a shift from type II to another response at low light intensity has also been described (Townsend and Risebrow 1982;

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Fig. 2. Capture rates as a function of prey density of Fontane cisco (*Coregonus fontanae*, black triangles and black lines) and vendace (*Coregonus albula*, grey triangles and grey lines) at (*a*) 5 lx, (*b*) 0.5 lx, and (*c*) 0.05 lx. Shown are all individual data and a curve fitted according to a Holling type II functional response, which was determined as the best-fit model. Capture rate increases with increasing prey density at all light intensities in both species. Fontane cisco showed a significantly lower functional response compared with vendace at 5 lx ($P = 0.003$), but not at 0.5 lx ($P = 0.051$) and 0.05 lx ($P = 0.129$).

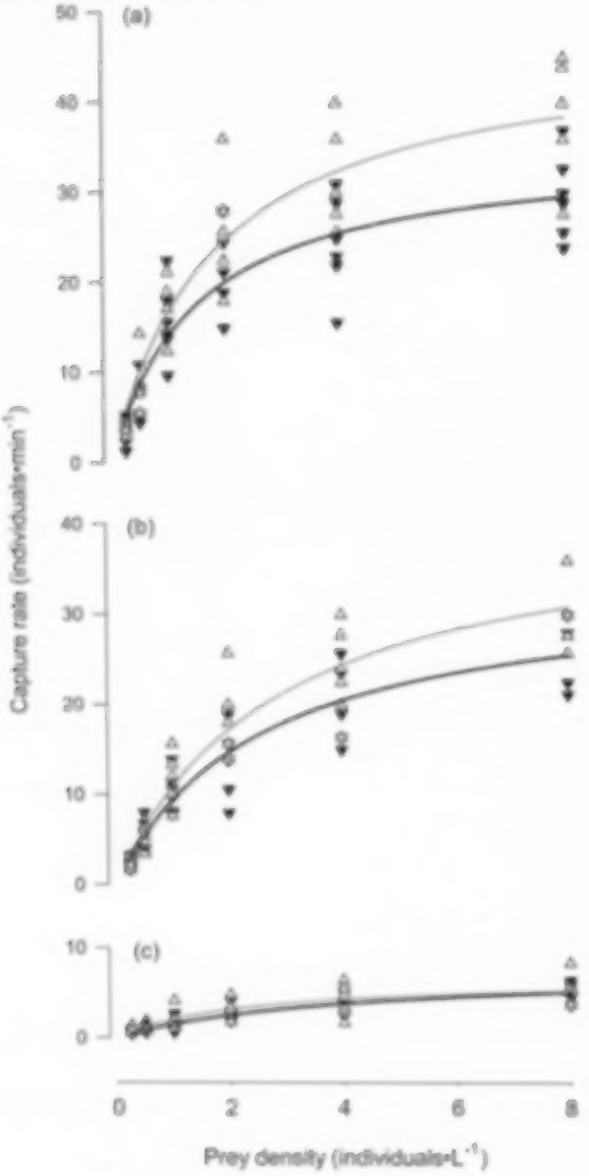
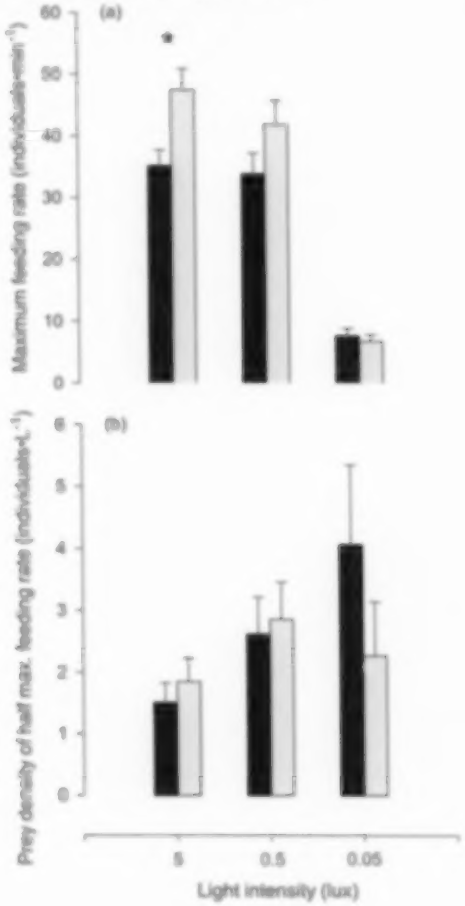


Fig. 3. (*a*) Maximum feeding rates and (*b*) prey densities of half maximum feeding rates of Fontane cisco (*Coregonus fontanae*, black bars) and vendace (*Coregonus albula*, grey bars) at 5, 0.5, and 0.05 lx. Shown are parameter estimates of the functional response type II fits and their standard errors. Maximum feeding rate significantly increases between 0.05 and 0.5 lx, whereas differences in prey density of half maximum feeding rate are not significant. Interspecies differences are significant only for the maximum feeding rate at 5 lx, as indicated by an asterisk.



Koski and Johnson 2002). However, the type II function is most frequently observed in planktivorous fishes (Jeschke et al. 2004). It has to be mentioned that different approaches for analyzing functional response data have been proposed (see Juliano 2001).

The two planktivorous coregonines of Lake Stechlin showed a marginal divergence in their feeding behaviour. Significant differences in functional response were only found at the highest light intensity and when associated with high food density. At all other light and food conditions investigated in this study, the species showed no difference in functional response. The combination of high food abundance and relatively high light intensity seems to represent a rather extreme condition. We re-examined field data based on zooplankton samplings from Lake Stechlin (Helland et al. 2007) and computed a mean zooplankton density for the most relevant prey species of the coregonines, including daphnids, small cladocerans, and copepods

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Table 4. Estimates and standard errors for parameters of the type II functional responses for both species at all three light intensities.

	Coregonus albula			*Coregonus fontanae*			Comparison	
	n	Estimate	SE	*n*	Estimate	SE	df	P
5 lx								
β_1	6	47.43	3.47	6	35.16	2.58	10	2.839
β_2	6	1.85	0.37	6	1.51	0.32	10	0.699
0.5 lx								
β_1	6	41.85	3.82	6	33.84	3.38	10	1.568
β_2	6	2.86	0.60	6	2.62	0.60	10	0.284
0.05 lx								
β_1	5	6.77	1.05	5	7.66	1.18	8	0.563
β_2	5	2.27	0.87	5	4.06	1.29	8	1.151

Note: The only significant difference between the species was found for β_1 at 5 lx. β_1, maximum consumption rate (individuals·min^{-1}); β_2, prey density of half maximum consumption rate; *n*, total number; SE, standard error; df, degrees of freedom.

of about 2.5 individuals·L^{-1} below 10 m depth. High food abundances of 8 individuals·L^{-1} or more in these depths are found only for some weeks during summer. Further, hydroacoustic surveys in the lake suggest that the fish feed in depths where light intensity is seldom at a level of 5 lx or above. The species stay below 35 m depth during day and migrate to lower depth during crepuscular light and at nighttime, so that foraging is generally associated with lower light intensities (S. Busch and T. Mehner, unpublished data). Thus, because of the migration pattern, a combination of high light intensity and high food density can be assumed to be very unlikely (i.e., for the most relevant environmental conditions found in situ, the species do not differ considerably with respect to their feeding efficiency). However, vendace feeding was slightly more efficient than that of the deeper living Fontane cisco at the combination of the highest light intensity and the highest food level, suggesting a beginning or ongoing segregation in their functional response along the light gradient.

Light intensity had a major influence on the feeding behaviour in both species, with declining efficiency down to a threshold between 0.05 and 0.005 lx. Generally, it is in accordance with many other studies on fish feeding behaviour that capture success is reduced as light intensity decreases. However, ongoing feeding activity of fishes that usually use vision for prey canture has been reported under light conditions approaching darkness in some species (e.g., Janssen 1978; Townsend and Risebrow 1982; Pekcan-Hekim and Horppila 2007). Hence, fishes might rely on nonvisual senses, for example the mechanosensory lateral line system, to locate and attack prey under light conditions where vision is ineffective (Janssen and Corcoran 1993; Hölker and Thiel 1998; Liang et al. 1998). The lack of feeding activity at the lowest light intensity (0.005 lx) investigated in this study suggests that the coregonines are unable to feed when light intensity approaches zero. However, we have to note that an effect of light spectrum on foraging behaviour cannot be completely ruled out. Hence, the described influence of light intensity on the feeding efficiency may possibly be confounded by an effect of color spectrum. This is contrasted by the fact that the change of color temperature during the experiments was associated with relatively minor changes in transmitted wavelength, since the color filter we used had a transmission characteristic of 50%–65% at 400–500 nm wavelengths but only 5%–10% in the 550–700 nm range, leaving mainly the blue and violet wavelengths just like clear water in depths of 15 m and more. Moreover, feeding activity of coregonines is generally discussed to be strongly influenced by light intensity and its diel and seasonal patterns. Dabrowski and Jewson (1984) showed that the feeding activity of Irish pollan larvae (*Coregonus pollan*) was strongly determined by light intensity and Gjelland et al. (2004) reported that feeding activity of pelagic common whitefish (*Coregonus lavaretus*) and vendace was more strongly correlated to light intensity than to circadian rhythms.

The diel vertical migration of the Lake Stechlin coregonines may be explained by the bioenergetics efficiency, the feeding opportunity, or the predator avoidance hypotheses (Scheuerell and Schindler 2003). If the latter applies, this would emphasize the importance of feeding at low light intensities to avoid predation, since yellow perch (*Perca flavescens*) as the most abundant predator species in the lake usually forage in shallow water at moderate to high light levels. However, hydroacoustic surveys from Lake Stechlin suggest that the seasonal variation of depth distribution of both species is more strongly correlated to temperature than to water transparency, and thus the migration patterns of these coregonines are only weakly related to the light regime and the occurrence of the most important food resources and potential predatory fish (Mehner et al. 2007). Hence, there is only little support for the predator avoidance as well as the feeding opportunity hypotheses, favoring the bioenergetics efficiency hypothesis to explain diel vertical migrations and putting forward temperature as the ultimate abiotic factor driving species' vertical orientation.

Our results clearly demonstrate a significant influence of food density and light intensity on capture rates in both species. Since they show an ecological segregation with respect to their vertical distribution within the pelagic habitat (Helland et al. 2007), where light intensity and food density decline continuously with depth (Mehner et al. 2005), these gradients induce severe species-specific consequences with respect to energy budgeting. The difference in average population depth imposes energetic disadvantages on the deeper-living Fontane cisco, because this species encounters lower food abundances under less favorable light conditions compared with sympatric vendace without showing any compensatory mechanism via enhanced feeding efficiency. This conclusion is supported by growth analysis (Schulz and Freyhof 2003) showing that individuals of Fontane cisco have a lower growth rate compared with vendace. That raises the question how this species is able to sustain a life-supporting energy budget under such unfavorable conditions and high exploitative competition with vendace.

The cooler temperatures at greater depths reduce the metabolic requirements considerably. An average temperature difference of only 1 °C means 7%–10% less metabolic costs for Fontane cisco. Furthermore, it appears that this species has adapted to the unfavorable conditions by reducing its standard metabolic rate. We found substantially lower standard costs in Fontane cisco compared with vendace over the

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whole range of environmental temperatures (Ohlberger et al. 2008). Although the relative amount of energy for metabolism of dwarf fish could be higher (Trudel et al. 2001), dwarfism per se might also be an adaptive strategy to living in deeper waters, instead of being its consequence, since smaller body size means on average less total energy expenditure for maintenance. Svärdson (1979) argued that in a situation of reduced food ration due to competition between pelagically living sympatric forms, the "dominated", inferior population could have a better energy budget if it withdraws to deeper and cooler waters. Further, the lower energy costs of smaller fish inspired Hamrin and Persson (1986) to base their hypothesis about the oscillation cycles in single vendace populations on a principle of competition between age classes. They argued that small vendace experience a competitive advantage compared with larger fish during periods of low food abundance because of their lower metabolic requirements.

In summary, in contrast with our hypothesis, the ecological segregation between the co-occurring species is not facilitated by differences in feeding efficiency, since their functional response curves barely differed within the natural range of environmental conditions. Instead, together with a study on the species' feeding preference, our data suggest that these coregonines are functionally similar, coexisting planktivores that only use slightly different feeding niches within their pelagic environment. We conclude that species' coexistence may be facilitated by alternative energetic strategies and recommend this as a promising field for future research.

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Coupling stable isotopes with bioenergetics to evaluate sources of variation in organochlorine concentrations in Baltic salmon (*Salmo salar*)

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Abstract: Atlantic salmon (*Salmo salar*) collected from three locations around the northern Baltic Sea in 2003–2004 showed large spatial and individual variation in their organochlorines (OCs) (polychlorinated dibenzo-*p*-dioxins and furans and polychlorinated biphenyls). This variation could be explained only partly by their size or sea age. The variability arose from the differences in salmon diet, trophic position, and prey OC concentrations and lipid content. A salmon bioenergetics accumulation model was used to evaluate the contribution of salmon growth and their diet to the observed individual variation in OC content. Our model revealed that the contribution of three main prey species in the OC accumulation of salmon varied markedly between the study areas. Amount of lipids in salmon explained a large proportion of their OC concentration. However, trophic position of salmon calculated from the $\delta^{15}N$ values explained almost 80% of the variation in lipid-normalized OC concentrations. In the Gulf of Finland, where OC concentrations of salmon were highest, their prey species had the highest OC concentrations and trophic positions. Higher OC concentrations in the Gulf of Finland might be related to elevated trophic positions caused by invasion of the predatory cladoceran *Cercopagis pengoi* in 1990.

Résumé : Des saumons (*Salmo salar*) récoltés à trois sites autour du nord de la Baltique en 2003–2004 affichent d'importantes variations spatiales et individuelles de leurs concentrations de composés organochlorés (OC; dibenzo-*p*-dioxines polychlorées et furanes et biphényles polychlorés). La taille des poissons et le temps passé en mer n'expliquent que partiellement cette variation. La variabilité provient de différences dans le régime alimentaire et la position trophique des saumons, de même que des concentrations d'OC et de lipides de leurs proies. Un modèle bioénergétique d'accumulation chez le saumon a servi à évaluer la contribution de la croissance et du régime alimentaire des saumons à la variation individuelle d'OC observée. Notre modèle révèle que les contributions des trois espèces principales de proies à l'accumulation d'OC chez les saumons varient considérablement d'une zone d'étude à l'autre. La quantité de lipides chez les saumons explique une proportion importante de leur concentration d'OC. Cependant, la position trophique des saumons calculée d'après les valeurs de $\delta^{15}N$ explique presque 80 % de la variation des concentrations normalisées d'OC dans les lipides. Dans le golfe de Finlande où les concentrations d'OC chez les saumons sont les plus grandes, les espèces de proies possèdent les concentrations d'OC les plus fortes et occupent la position trophique la plus élevée. Les concentrations plus élevées d'OC dans le golfe de Finlande peuvent être reliés à des positions trophiques supérieures résultant de l'invasion du cladocère prédateur *Cercopagis pengoi* en 1990.

[Traduit par la Rédaction]

Introduction

Baltic Sea fish, in general, possess very high concentrations of organochlorine compounds (OCs), such as polychlorinated dibenzo-*p*-dioxins and furans (PCDD/Fs) and polychlorinated biphenyls (PCBs). PCDD/F and PCB concentrations in fatty fish species such as Baltic salmon (*Salmo salar*), Baltic herring (*Clupea harengus membras*), and Baltic sprat (*Sprattus sprattus*) frequently exceed the present EU limits for foodstuffs of 8 pg $WHO_{PCDD/F-PCB}$-TEq·g^{-1} (Isosaari et al. 2006), henceforth referred to as $WHO_{n-d/l}$-TEq. However, there is considerable spatial and individual variation in OC concentrations of Baltic fish (Berglund et al. 2001; Isosaari et al. 2006; Parmanne et al. 2006). Spatial and temporal variations in food web biomagnification, from phytoplankton through zooplankton to fish, are affected by anthropogenic loading and environmental factors, as well as by differences in community structure

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and function. There have been some attempts to assess origin of this variation for Baltic salmon (Vuorinen et al. 1997; Berglund et al. 2001; Vuorinen et al. 2002), but the underlying ecological processes are still poorly understood.

One hypothesis is that the high individual variability in OC concentrations in salmonine reflects variability in the diets of individual fish (Madenjian et al. 1994). This could arise through regional and temporal differences in prey fish abundance or through optimal foraging preferences exhibited by individuals. About 99% of the total body mass and therefore nearly all of the toxicants in salmon are accrued during feeding migration from the feeding areas (Berglund et al. 2001). There are three main feeding areas of salmon in the Baltic Sea: the Bothnian Sea, the Baltic Proper, and the Gulf of Finland (Ikonen 2006; International Council for the Exploration of the Sea (ICES) 2006). Most postsmolt salmon from the rivers draining into the Bothnian Sea migrate to the main feeding areas in the Baltic Proper, but each year, some proportion remains and feeds in the Bothnian Sea, especially if the prey fish stocks are strong (Kallio-Nyberg et al. 1999; Salminen et al. 2001). Postsmolts originating from the Gulf of Finland stocks also feed to a substantial extent in the Main Basin, but at the same time, populations from the other sea areas may feed in the Gulf of Finland. Availability of the salmon prey fish species varies markedly in each of the feeding sites (Ikonen 2006; ICES 2006), which will affect the proportion of each prey species in the diet. According to stomach analyses, sprat, herring, and threespine stickleback (*Gasterosteus aculeatus*) dominate (>90%) the diet of Baltic salmon (Karlsson et al. 1999; Hansson et al. 2001). In the Baltic Proper, the diets consist mostly of sprat, whereas in the Bothnian Sea, salmon feed mainly on herring (Karlsson et al. 1999; Hansson et al. 2001; Salminen et al. 2001). However, large individual variability in prey fish proportion and size has been observed. Such differences in the salmon diet in conjunction with high observed intra- and inter-species variability in prey fish OC concentration (Vuorinen et al. 2002; Parmanne et al. 2006) can be expected to affect the OC concentration exhibited by salmon feeding populations, as well as by individuals.

Stable isotope analysis (SIA) is an excellent tool to link consumers to their diet and contaminant sources (Cabana and Rasmussen 1994). Use of stable isotopes of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) as natural markers in ecological and ecotoxicological studies is based on laboratory experiments (DeNiro and Epstein 1978), which have shown stable isotopes to fractionate in trophic transfer in a rather predictable manner. $\delta^{13}C$ shows only slight or no enrichment relative to the diet and therefore has been used to describe dietary sources, whereas $\delta^{15}N$ values are enriched by about 3‰ between diet and consumer and have been used to describe the trophic level of the consumer. Based on these principles, SIA has been used to explain variation in consumer contaminant concentrations in several applications (Jardine et al. 2006). Stable N isotopes enable determination of trophic level, which can be further used to explain variation in contaminant concentration between populations. Stable isotopes (usually C and N) can also be used to obtain dietary proportions for investigated species (Phillips 2001), and SIA combined with bioenergetics accumulation modelling makes it possible to study movements of harmful substances in the food web (Caut et al. 2006). The bioenergetics modelling is based on the analysis of growth and metabolic rates of individuals, but because of the hierarchic structures of energy budgets, it also describes efficiently the trophic transfer and thus links different levels of biological organization (Kooijman 2000). Bioenergetics bioaccumulation models have recently been used to study bioaccumulation of OCs in Baltic herring (Kiljunen et al. 2007; Peltonen et al. 2007).

Fig. 1. The three study areas in the northern Baltic Sea and their International Council for the Exploration of the Sea subdivisions (indicated in parentheses).

This study evaluates the origins of spatial and individual variation in OC concentrations in Baltic Sea salmon using bioenergetics bioaccumulation models and SIA of C and N. The purposes of the study are (*i*) to apply and validate an individual-based bioenergetics accumulation model for Baltic Sea salmon, (*ii*) to use dietary information provided by SIA in the bioenergetics accumulation model to quantify the contribution of each prey species to PCB and PCDD/F accumulation in Baltic salmon, and (*iii*) to determine the extent to which trophic level (determined by SIA) or other factors (fat content, weight, age, etc.) could explain the observed variation in OC concentrations in individual salmon.

Material and methods

Sampling and sample preparation

A total of 25 salmon where caught with drift nets from the three different open-sea study areas (Fig. 1) during their feeding migration from 24 November to 2 December in 2003 and 2004 and immediately frozen after collection. Salmon were thawed in the laboratory, and total length and weight were measured. Scales above the lateral line and below the dorsal fin were removed for aging and determination

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of back-calculated growth. Back-calculated fish total length at annulus i (L_i) was calculated by Dahl–Lea model:

$$(1) \quad L_i = L_c(S_i/S_c)$$

where L_c is fish total length at capture, S_i is scale radius to annulus i, and S_c is total scale radius. The model has been shown to be appropriate for back-calculating salmon total lengths (Heidarsson et al. 2006). Salmon weights where calculated with the length–weight relationship obtained from the database of the Finnish Game and Fisheries Research Institute (information available from Tapani Pakarinen, FGFRI, P.O. Box 2, FIN-00791 Helsinki, Finland). Fish were opened for removal of gut contents and sex determination. The salmon carcases were then individually homogenized using an industrial blender. Homogenates were thoroughly mixed and samples were taken for OC analysis and SIAs.

Salmon prey species from the same areas were obtained from commercial trawl catches between October and December 2003 (herring and sprat), except for threespine sticklebacks, which were collected between April and June 2004. After collection, herring and sprat total length (nearest millimetre), weight (nearest milligram), and sex were determined and otoliths were removed for aging. Fish were then individually frozen. Herring and sprat were aged from otolith cross sections prepared using a high-precision cutoff machine (Struers Accutom 50, Copenhagen, Denmark) and stained with neutral red. Five age-specific pooled samples (1, 2, 3, 6, and 10) for herring and three (1, 3, and 7) for sprat were created from each study site. Threespine sticklebacks were not aged but were pooled according to sampling year and specific location. Two threespine stickleback pools from the Bothnian Sea, one from the northern Baltic Proper, and four from the Gulf of Finland were made. All of the pools were further homogenized for OC analysis and SIAs.

To generate stable isotope baselines (based on primary consumers) for each study site, 16 bulk zooplankton samples were collected on the research vessels *Muikku* and *Aranda* during August 2003 and 2004 by hauling a plankton net (mesh size 200 μm) from the bottom to the surface. Immediately after collection, zooplankton samples were frozen to –20 °C. In the laboratory, the samples were thawed and cladocerans were hand-picked from the samples. Because the biomass of cladocerans was generally very small, some of the samples were pooled. A total of five cladoceran samples from the Bothnian Sea and two each from the northern Baltic Proper and the Gulf of Finland were analyzed for stable isotopes.

OC and energy content analyses

At the Finnish National Public Health Institute (Kuopio, Finland), homogenized fish samples were freeze-dried and Soxhlet-extracted for 20 h with toluene, and fat content was measured gravimetrically from the extract. Purification of the samples and fractionation of the studied compounds were as described previously (Isosaari et al. 2002; Kiviranta et al. 2003). High-resolution gas chromatography – mass spectrometry was used to determine OC concentrations. The analytical procedure and quality control have been described in more detail by Isosaari et al. (2006). The recoveries of the individual internal standards of PCDD/Fs and PCBs were determined by adding the recovery standards just before the mass spectral analyses; two recovery standards for PCDD/Fs (1,2,3,4-TCDD and 1,2,3,7,8,9-HxCDD) and one for each non-*ortho*-PCB and mono- and di-*ortho*-PCB (^{13}C-labelled PCB 60 and ^{13}C-labelled PCB 159, respectively) were used. Recoveries were all >60%. Limits of quantification corresponded to a signal to noise ratio of 3:1 in the mass spectrometry chromatograms. Limits of quantification for individual congeners were 0.1–1.5 pg·g^{-1} for PCDD/Fs and for non-*ortho*-PCBs and 0.002–0.01 pg·g^{-1} for mono- and di-*ortho*-PCBs. Blanks covering the whole analytical procedure were analyzed along with the samples (one blank for every five samples). Blank samples did not indicate any problems with laboratory or cross-sample contamination. The method uncertainty estimate is based on an in-house fish oil control sample and several international quality control studies in which the laboratory has participated. The matrixes in these studies have included milk, egg, meat, oil and fat, and fish samples (Lindström et al. 2000; Becher et al. 2001; Smástuen Haug and Becher 2006). The laboratory is an accredited testing laboratory (No. T077) in Finland (current standard EN ISO/IEC 17025). The scope of accreditation includes PCDD/Fs, non-*ortho*-PCBs, and PCBs from environmental and food samples. The coefficients of variation of $WHO_{PCDD/F}$-TEq and WHO_{PCB}-TEq in replicate in-house fish oil control sample analyses were <10%, and these, together with the results from international quality control studies, gave the method uncertainties of TEqs below 20%. Concentrations of 17 PCDD/F and 12 PCB congeners (Table 1) were calculated as "lower bound" values (concentrations of nondetected congeners are set to zero) per wet weight of fish muscle tissue (note that in "upper bound" concentrations, nondetected congeners are set as their limits of quantification). The toxic equivalent concentrations (WHO-TEq) for both PCDD/Fs and PCBs were calculated by summing concentrations of the toxic congeners multiplied by their toxic equivalent factor values (Table 1) according to van den Berg et al. (1998). Results are presented as pg WHO_{total}-TEq·g wet weight^{-1}, which is the sum of $WHO_{PCDD/F}$-TEq and WHO_{PCB}-TEq concentrations. In the European Union, this unit has a maximum permissible level of 8 pg·g^{-1} for fish and fisheries products (European Community 2006).

A small part of each homogenized and freeze-dried salmon, herring, and sprat samples was further analyzed for energy density using an adiabatic bomb calorimeter (Automatic MK 200) according to Raymond et al. (1957) at the Finnish Game and Fisheries Research Institute (Helsinki, Finland).

SIA and calculation of trophic position

Stable isotope samples from whole fish and cladocerans were freeze-dried to a constant weight. Samples were ground to fine powder using either a freezer mill (6750 SPEX; CertiPrep Inc., New Jersey, USA) or a mortar and pestle and stored frozen in glass vials prior to isotope analysis. Stable isotopes of C and N were analyzed at the University of Jyväskylä, Finland, using a Carlo Erba Flash EA1112 elemental analyser connected to a Thermo Finnigan DELTA^{plus} Advantage continuous-flow stable isotope – ratio

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Table 1. Mean (±SD) organochlorine concentrations of Baltic salmon (*Salmo salar*) and its prey species (Baltic herring (*Clupea harengus membras*), sprat (*Sprattus sprattus*), and threespine stickleback (*Gasterosteus aculeatus*)) sampled from three study areas in the Baltic Sea.

	Congener concentration ($pg \cdot g^{-1}$)							
Congener	Salmon	Herring	Sprat	Threespine stickleback	AE_c	AE_p	TEF	log K_{ow}
PCBs								
PCB 123	940±350	310±240	220±130	210±190	0.74	0.85±0.27	0.00010	6.46[f]
PCB 118	11 900±5390	3530±2990	2460±1570	2230±1840	0.78	0.90±0.34	0.00010	6.61[d]
PCB 114	200±120	60±50	400±300	40±30	0.80[a]	0.83±0.47	0.00050	6.39[f]
PCB 105	4420±2320	1190±1020	870±560	710±530	0.76	0.95±0.39	0.00010	6.57[d]
PCB 167	490±260	190±130	150±80	140±120	0.80[a]	0.73±0.38	0.00001	6.92[c]
PCB 156	1900±860	500±440	360±230	370±350	0.79	0.96±0.57	0.00050	6.84[c]
PCB 157	430±220	110±110	80±50	60±40	0.80[a]	0.95±0.54	0.00050	6.84[c]
PCB 189	180±70	50±40	40±20	30±30	0.80[a]	1.10±0.67	0.00010	7.30[c]
PCB 81	1.94±0.86	0.74±0.32	0.72±0.19	4.58±5.91	0.97	0.61±0.33	0.00010	6.14[c]
PCB 77	98.91±41.96	26.47±8.31	51.41±29.86	72.55±104.35	0.87	0.75±0.19	0.00010	6.14[c]
PCB 126	41.11±11.99	15.31±8.16	15.60±8.08	11.00±5.96	0.92	0.72±0.14	0.10000	6.60[c]
PCB 169	14.93±4.83	6.40±5.14	4.06±2.28	1.36±0.58	0.84	0.83±0.38	0.01000	7.06[c]
WHO_{PCB}-TEq	7.28±1.94	2.45±1.53	2.21±1.21	1.67±1.06				
PCDD/Fs								
2378-TCDF	4.87±1.79	2.40±1.12	2.35±0.90	2.88±1.59	0.64	0.64±0.16	0.10000	6.10[c]
2378-TCDD	0.41±0.11	0.20±0.14	0.14±0.08	0.16±0.08	0.72	0.71±0.12	1.00000	6.42[c]
12378-PeCDF	0.65±0.30	0.67±0.59	0.40±0.21	0.20±0.14	0.58	0.36±0.10	0.05000	7.26[c]
23478-PeCDF	7.27±3.09	5.10±4.93	2.50±1.33	0.88±0.66	0.56	0.52±0.17	0.50000	6.50[c]
12378-PeCDD	0.82±0.29	0.60±0.56	0.28±0.18	0.17±0.09	0.64	0.50±0.10	1.00000	6.64[c]
123478-HxCDF	0.11±0.05	0.20±0.20	0.13±0.06	0.06±0.04	0.33[b]	0.20±0.09	0.10000	7.00[c]
123678-HxCDF	0.15±0.07	0.28±0.28	0.18±0.11	0.06±0.04	0.32	0.19±0.07	0.10000	7.20[b]
234678-HxCDF	0.14±0.06	0.26±0.24	0.21±0.15	0.08±0.04	0.33[b]	0.16±0.06	0.10000	6.94[b]
123789-HxCDF		0.01±0.01			0.33[b]		0.10000	7.13[b]
123478-HxCDD	0.02±0.03	0.06±0.05	0.03±0.02	0.02±0.02	0.33[b]	0.35±0.45	0.10000	7.80[c]
123678-HxCDD	0.41±0.16	0.61±0.70	0.30±0.21	0.11±0.08	0.34	0.23±0.07	0.10000	7.64[b]
123789-HxCDD	0.00±0.01	0.06±0.05	0.05±0.04	0.02±0.02	0.33[b]	0.01±0.05	0.10000	7.61[e]
1234678-HpCDF	0.03±0.04	0.10±0.10	0.13±0.20	0.06±0.04	0.24[b]	0.05±0.08	0.01000	7.40[c]
1234789-HpCDF		<0.00±<0.00			0.24[b]		0.01000	7.62[b]
1234678-HpCDD	0.00±0.02	0.07±0.03	0.09±0.05	0.17±0.19	0.24	0.01±0.04	0.01000	8.00[c]
OCDF		0.03±0.06	0.03±0.05		0.00		0.00010	8.00[c]
OCDD	0.03±0.08	0.15±0.11	0.07±0.08	0.36±0.40	0.00	0.03±0.07	0.00010	8.20[c]
$WHO_{PCDD/F}$-TEq	5.47±1.99	3.77±3.40	2.01±1.06	1.11±0.67				

Note: Experimentally determined assimilation efficiencies by Isosaari et al. (2005) (AE_c) and predicted assimilation efficiencies (AE_p) using bioenergetics model from this study and toxic equivalent factors (TEF) and *n*-octanol–water partitioning coefficients (log K_{ow}) of each congener are also given.

[a]Average assimilation efficiency of all PCB congeners measured by Isosaari et al. (2005).

[b]Average assimilation efficiency of PCDD/F congeners having same number of chlorine atoms.

[c]Hansen et al. (1999).

[d]Türker Saçan and Inel (1995).

[e]Mackay et al. (1997).

[f]Zheng et al. (2003).

[g]Sharma et al. (2007).

[h]Chen et al. (2001).

mass spectrometer. Results are expressed using the standard δ notation as parts per thousand (‰) difference from the international standards. The reference materials used were internal standards of known relationship to the international standards of Vienna Pee Dee belemnite (for C) and atmospheric N_2 (for N). Dried pike white muscle was used as an internal working standard and two replicate standards were run repeatedly after every 10 samples in each sequence. Internal precision was always better than 0.2‰ based on the standard deviation of replicates of the standards. Sample analysis also yielded percent C and N from which C–N ratios (by weight) were derived. Because lipids are known to be ^{13}C-depleted (have lower $\delta^{13}C$) relative to other major tissue constituents (DeNiro and Epstein 1978; Focken and Becker 1998; Thompson et al. 2000), C isotope ratios were lipid-normalized (indicated here as $\delta^{13}C'$) using the C–N ratio according to Kiljunen et al. (2006).

When comparing stable isotope values of different systems in ecotoxicological studies, it is necessary to standardize $\delta^{15}N$ values of the organism relative to trophic position

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(Jardine et al. 2006). Reliable determination of trophic position is based on good baseline estimates. In this study, we used cladocerans as baseline organisms. Because the $\delta^{15}N$ values of short-lived aquatic primary consumers are spatially and temporally variable (Rolff 2000; Rolff and Elmgren 2000; Syväranta et al. 2006), baseline estimates are preferably taken from long-lived primary consumers such as mussels and snails (Vander Zanden et al. 1997), which integrate temporal variability in primary consumer $\delta^{15}N$ signatures. However, in deep pelagic systems like the Baltic Sea, use of such organisms is questionable, as the basal value in the profundal might be very different from that in the pelagic because of microbial degradation of settling organic matter (Velinsky and Fogel 1999). Besides, several areas of the Baltic lack bottom fauna due to oxygen depletion at the bottom, which is another factor to support application of cladocerans as baseline organisms. Trophic position (TP) was calculated for each salmon individual using the equation of Vander Zanden et al. (1997):

$$(2) \quad TP_{organism} = (\delta^{15}N_{organism} - \delta^{15}N_{primary\ consumer})/\Delta^{15}N + 2$$

where $\delta^{15}N_{organism}$ and $\delta^{15}N_{primary\ consumer}$ are measured $\delta^{15}N$ values of salmon and cladocerans. $\Delta^{15}N$ is enrichment of $\delta^{15}N$ for one trophic level, which was here assigned to be 2.9 based on whole-fish $\Delta^{15}N$ values reviewed by Sweeting et al. (2007).

Bioenergetics accumulation model and model validation

To estimate the contribution of prey species to salmon PCB and PCDD/F accumulation, we constructed site-specific bioenergetics accumulation models that were based on the model of Hanson et al. (1997) and that used model parameters from Beauchamp et al. (1989). The model was validated for salmon consumption (C) by comparing published experimental consumption estimates by Koskela et al. (1997*a*, 1997*b*) with those estimated by the bioenergetics model. The bioenergetics model was based on a mass balance energy budget in which energy consumed by a predator (C) is equal to the sum of metabolism (R), waste losses (E), and growth (G) (Winberg 1960). The food consumption estimates were calculated in grams of prey per gram of salmon and were converted to energy density (joules per gram).

Salmon diet was assumed to consist only of herring, sprat, and threespine stickleback (Hansson et al. 2001). Quantitative dietary information provided by the stable isotopes was intended for use as input data in the bioenergetics accumulation model. Unfortunately, overlap between prey species was too high to calculate sensible estimates of prey species proportion in individual salmon diet using stable isotope mixing models (Phillips 2001). Therefore, the published dietary proportions (Hansson et al. 2001) were used in the bioenergetics model. In the Bothnian Sea, all individuals were set to feed 62% on herring, 26% on threespine stickleback, and 12% on sprat. In the northern Baltic Proper, prey species proportions were set to 70%, 25%, and 5% for sprat, herring, and threespine stickleback, respectively. Such data were not available for the Gulf of Finland; however, our stable isotope data suggested similar proportions as in the Bothnian Sea. Species-specific information on salmon prey length were applied from previous Baltic salmon stomach analyses (Sture Hansson, Department of Systems Ecology, Stockholm University, SE-106 91 Stockholm, Sweden, personal communication (2004)) to generate models for salmon prey length assuming a log-normal distribution of residual errors (ε) with μ of 0 and SE^2.

$$(3) \quad H_L = 3.351 Sa_L e^{\varepsilon},\ \varepsilon \sim N(0, 0.199^2)$$

$$(4) \quad S_L = 8.7703 Sa_L e^{\varepsilon},\ \varepsilon \sim N(0, 0.167^2)$$

$$(5) \quad Sb_L = 3.017 Sa_L e^{\varepsilon},\ \varepsilon \sim N(0, 0.164^2)$$

where H_L, S_L, Sb_L, and Sa_L are herring, sprat, threespine stickleback, and salmon total lengths, respectively. The predator length – prey length models were corrected for the length underestimation in the stomach analyses (Johansen et al. 2004) and preservation shrinkage (Ajah and Nunoo 2003), which were assumed to be 10% in total.

Based on our own analyses, mean herring energy densities in the Bothnian Sea, northern Baltic Proper, and Gulf of Finland were set to 6228, 5812, and 4871 J·g wet weight^{-1}, respectively, and sprat energy densities were set to 7829, 6860, and 6382 J·g wet weight^{-1}, respectively. An energy density of 4967 J·g wet weight^{-1} was used for threespine stickleback (Meakins 1976; Smith and Wootton 1995) at all study sites. Salmon energy density was set according to the original parameters (Beauchamp et al. 1989), as their model fit well to our own salmon energy density data. The water temperatures used in calculations were average monthly temperatures measured in the Bothnian Sea and the Gulf of Finland in 1990–2000 from the top 10 m (Water Quality Register, Finnish Environment Institute: www.ymparisto.fi/default.asp?contentid=187886&lan=en#a7). For the northern Baltic Proper, temperatures from the Gulf of Finland were used.

Daily consumption estimates were further used to predict the daily uptake of 17 PCDD/F and 12 PCB congeners into salmon tissue from the food consumed:

$$(6) \quad X_c = C_c AE_c$$

where X_c is the assimilation of a congener into fish tissue, C_c is the total amount of a congener in food consumed (mass of prey consumed (g) multiplied by the prey congener concentration (pg·g^{-1})), and AE_c is the assimilation efficiency of the congener (Hanson et al. 1997). Congener-specific exponential models for herring and sprat lengths (means of the pools analyzed for OCs) in relation to congener concentration were generated to estimate concentrations in particular sizes of salmon prey. Threespine stickleback OC concentrations were set as constant (mean concentration of the pools). All the calculations were done on a fresh weight basis. We assumed that salmon assimilates each PCDD/F and PCB congener at a congener-specific and constant rate. Most of the congener-specific assimilation efficiencies were derived from Isosaari et al. (2005) (Table 1). If the assimilation efficiency was not available for a certain congener, we used the average of the PCDD/F congeners with the same number of chlorine atoms, as accumulation

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Table 2. Mean (±SD) weight, WHO_{total}-TEq concentration, fat content, $\delta^{15}N$ values, and lipid-normalized $\delta^{13}C$ ($\delta^{13}C'$) values of individual Baltic salmon (*Salmo salar*) and pooled Baltic herring (*Clupea harengus membras*), sprat (*Sprattus sprattus*), threespine stickleback (*Gasterosteus aculeatus*), and cladoceran samples.

Species	Weight (g)	WHO_{total}-TEq ($pg \cdot g^{-1}$)	Fat (%)	$\delta^{15}N$ (‰)	$\delta^{13}C'$ (‰)	C ($g \cdot g^{-1}$)	% in salmon WHO_{total}-TEq	n
Bothnian Sea								
Salmon	3718±686	12.8±1.5	16.7±0.7	12.4±0.3	–19.7±0.2	2.8±0.1		5
Herring	29±15	7.3±6.4	8.4±0.7	9.9±0.4	–20.4±0.5		75.1	5
Sprat	10±2	3.1±1.5	14.4±3.3	8.7±0.4	–20.2±0.3		8.5	3
Threespine stickleback		3.1±1.5	16.0±5.9	9.0±0.3	–20.7±0.5		16.4	2
Cladocerans				4.1±2.4	–24.0±0.8			5
Northern Baltic Proper								
Salmon	4106±1630	11.5±2.6	16.6±2.5	12.4±0.5	–18.7±0.3	2.8±0.1		
Herring	30±16	4.9±3.9	6.9±1.0	10.8±0.3	–20.3±0.3		17.0	5
Sprat	8±2	4.4±2.1	11.6±3.9	9.5±0.7	–19.5±0.2		80.4	3
Threespine stickleback		3.2	9.1	10.0	–20.2		2.6	1
Cladocerans				4.8±3.3	–23.4±0.9			2
Gulf of Finland								
Salmon	2655±1684	14.0±5.0	10.3±3.3	14.3±0.9	–17.9±0.4	3.5±0.2		10
Herring	18±8	6.5±5.0	4.6±1.0	11.6±0.6	–19.0±0.6		78.4	5
Sprat	8±2	5.2±3.2	9.5±1.8	10.0±0.4	–19.2±0.3		12.8	3
Threespine stickleback		2.5±1.8	7.4±3.9	11.2±0.8	–19.0±0.9		8.8	4
Cladocerans				4.5±0.6	–21.8±0.3			9

Note: Mean (±SD) food consumption (C, $g \cdot g^{-1}$) during sea migration (245–975 days) estimated by salmon bioenergetics model and proportion of each prey fish in salmon WHO_{total}-TEq concentration. Number of analyzed samples (n) for each of the three studied areas of the Baltic Sea is also given.

efficiency of PCDD/Fs decreases with increasing substitution (Lundebye et al. 2004). If assimilation efficiency was not available for a certain PCB congener, it was replaced with the mean of all of the available PCB assimilation efficiencies (0.8). The total uptake of each individual congener was calculated as a cumulative sum of the daily uptakes, and the final concentration estimates ($pg \cdot g^{-1}$) were calculated by dividing the total uptake by fish mass. To evaluate the suitability of congener-specific assimilation efficiencies experimentally determined by Isosaari et al. (2005) (AE_e) (Table 1), the predicted assimilation efficiencies (AE_p) (Table 1) were calculated by dividing the observed total amount of each congener in salmon individuals by the total uptake of congeners predicted by the bioenergetics model.

All of the calculations where done with time steps of 1 day. Because 99% of the total body mass and therefore nearly all of the toxicants of the salmon are accrued during the feeding migration (Berglund et al. 2001), the river phase was ignored and 1 May was assumed to be the first day of the sea feeding phase (postsmolt). Modelling was carried out on individuals from the postsmolt until the day of catching using back-calculated growth from the scales. Because of stochastic components in the prey size selection, the mean of 1000 simulations for each individual salmon ($n = 24$) was calculated. One salmon from the Gulf of Finland had to be removed from the model because of inaccuracy in age determination. The whole model, including contaminant calculations, was further validated by comparing the predicted concentration estimates from the bioenergetics accumulation model with the observed concentrations of PCDD/Fs and PCBs measured from the same individuals.

Statistical methods

The bioenergetics model was validated by comparing measured and estimated values and testing regression constant and slope against a 1-to-1 ratio. WHO_{total}-TEq values were lipid-normalized using analysis of covariance (ANCOVA) according to Hebert and Keenleyside (1995). The SPSS statistical software package (version 14.0.2; SPSS Inc., Chicago, Illinois) was used in statistical analyses with a criterion for significance of $p < 0.05$. Lipid-normalized data were nonnormally distributed and were $\log_{10}$-transformed before statistical analyses. Non-lipid-normalized data were found to be normally distributed and no transformation was necessary. Analysis of variance (ANOVA) was used in comparison between the sites and for species differences in OC concentration and isotope values, and then, Tukey tests were used for pairwise comparisons. If assumptions for ANOVA were not valid, the nonparametric Kruskal–Wallis test was used, and in the case of significant results, Mann–Whitney U tests were used in pairwise comparisons with a Bonferroni correction. Homogeneity of regression coefficients between sites ($\delta^{15}N$ versus WHO-TEq) was examined with the ANCOVA interaction term $\delta^{15}N \times$ study site.

Results

The measured Baltic salmon mean WHO_{total}-TEq concentrations (Table 2) markedly exceed EU limits (8 pg WHO_{total}-TEq$\cdot g^{-1}$) for fish and fishery products. The mean concentrations were highest in the Gulf of Finland. However, in the Gulf of Finland, a few individuals actually had concentrations slightly below the limit of 8 $pg \cdot g^{-1}$. Age structure of the samples between sites was not equal; therefore, intersite comparison of WHO_{total}-TEq concentrations

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Fig. 2. One-seawinter Baltic salmon (*Salmo salar*) WHO_{total}-TEq wet weight concentrations (open circles, measured values; solid circles, lipid-normalized values) in the three study areas. Significant differences between areas are indicated by different letters at the top.

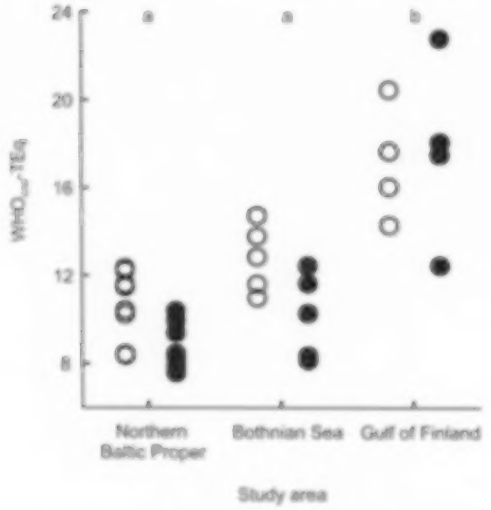


Fig. 3. Baltic salmon (*Salmo salar*) prey fish ((*a*) Baltic herring (*Clupea harengus membras*) and (*b*) sprat (*Sprattus sprattus*)) length in relation to their WHO_{total}-TEq concentrations indicated as curves and prey size percentage distribution in salmon diet (recalculated from Hansson et al. 2001) in the three study areas. Curves are exponential fits to five herring and three sprat pooled samples from each study area. Squares and solid line, northern Baltic Proper; diamonds and broken line, Bothnian Sea; circles and dashed–dotted line, Gulf of Finland.

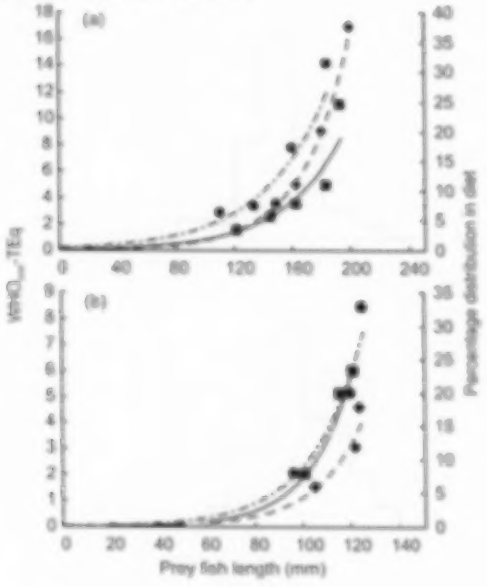
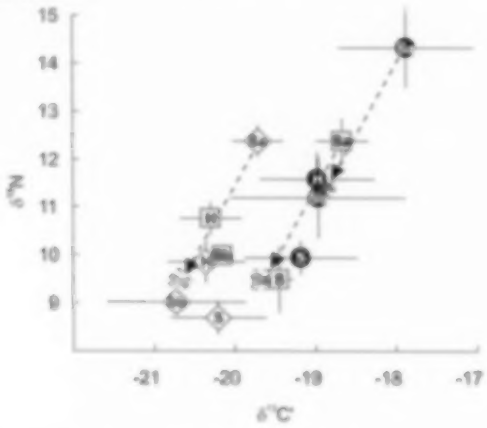


Fig. 4. $\delta^{15}N$ and lipid-normalized $\delta^{13}C$ values (±SD) of Baltic salmon (*Salmo salar*) (Sa) and its prey species (H, Baltic herring (*Clupea harengus membras*); S, sprat (*Sprattus sprattus*); Sb, threespine stickleback (*Gasterosteus aculeatus*)) from three study areas. Broken arrows and markers represent placement of salmon values after correction for trophic fractionation ($\delta^{15}N$ = 2.9, $\delta^{13}C'$ = 1). Diamonds, Bothnian Sea; squares, northern Baltic Proper; circles, Gulf of Finland.



was made only for one-seawinter individuals. Concentrations were significantly higher in the Gulf of Finland ($F_{[2]}$ = 16.584, $p < 0.001$) and also showed a higher degree of variability. No intersite difference in weight was observed ($\chi^2_{[2]}$ = 0.024, p = 0.988). Non-lipid-normalized WHO_{total}-TEq concentrations in one-seawinter individuals from the Gulf of Finland differed significantly from those from the Bothnian Sea (p = 0.008) and the northern Baltic Proper ($p < 0.001$), and variation was clearly higher in the Gulf of Finland than in the other sites (Fig. 2). Because WHO_{total}-TEq concentrations were associated with lipids and there was intersite difference in the lipid content of one-seawinter individuals ($\chi^2_{[2]}$ = 6.397, p = 0.041), WHO_{total}-TEq concentrations were ANCOVA lipid-normalized. Normalization did not change the interpretation of the results: the Gulf of Finland fish still had higher concentrations compared with those from the Bothnian Sea (p = 0.001) and the northern Baltic Proper ($p < 0.001$), and variation even increased compared with other sites (Fig. 2), which was due to markedly lower lipid content in fish in the Gulf of Finland than in fish in the Bothnian Sea and northern Baltic Proper (Table 2).

Lipid content of prey species was lowest in the Gulf of Finland, and prey species OC concentrations varied markedly between sites and species (Table 2). Mean herring OC concentrations were highest in the Bothnian Sea, but those of sprat were highest in the Gulf of Finland (Table 2). However, those herring and sprat length classes most frequent in the salmon diet had higher WHO_{total}-TEq concentrations in the Gulf of Finland (Fig. 3) than in the northern Baltic Proper and the Bothnian Sea.

Salmon, its prey species, and study areas were separated by the stable isotopes, although there was considerable overlap (Fig. 4). In all study areas, salmon had the highest $\delta^{15}N$ values. Of the prey species, herring gave the highest and sprat the lowest $\delta^{15}N$ values, while threespine stickleback

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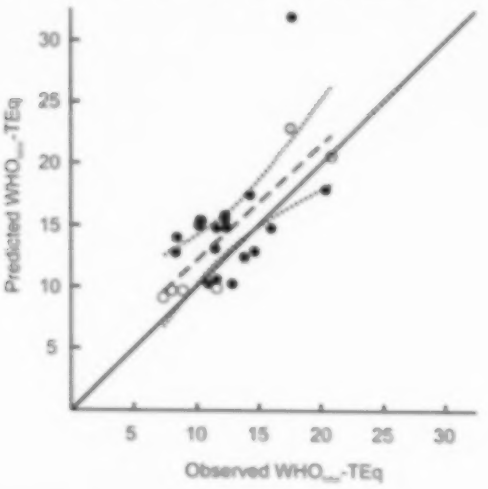
Fig. 5. Predicted (calculated using bioenergetics accumulation model) versus observed WHO_{total}-TEq concentrations (pg·g^{-1}) of 24 Baltic salmon (*Salmo salar*). The solid line shows the 1-to-1 ratio for predicted and observed values; the broken line and dotted lines indicate the fitted linear relationship and the 95% confidence limits, respectively. Open circles, 0+ seawinter salmon; solid circles, 1+ seawinter salmon; shaded circles, 2+ seawinter salmon.

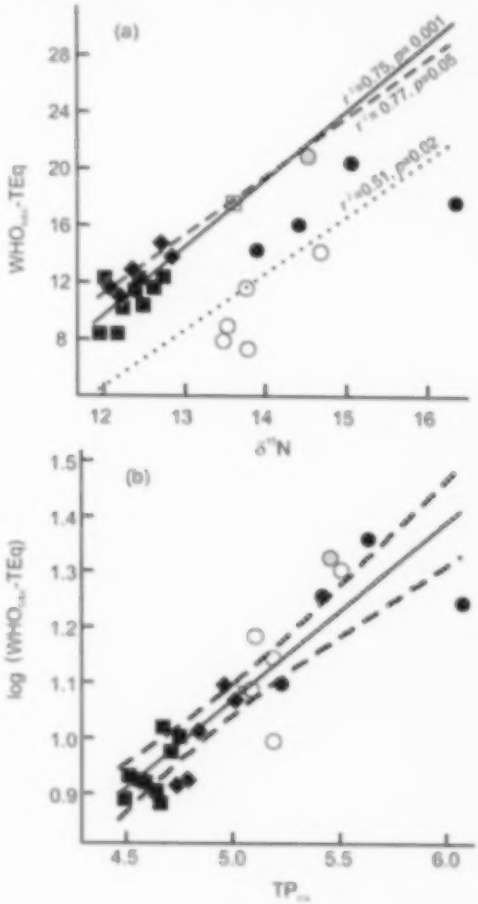


values were intermediate. Stable isotope values of the Baltic salmon differed significantly between areas ($\delta^{13}C$, $F_{[2]} = 44.81$, $p < 0.001$; $\delta^{15}N$, $F_{[2]} = 26.38$, $p < 0.001$). The Gulf of Finland salmon were distinct from those from the other sites for both isotopes ($p < 0.001$), whereas the same species in the Bothnian Sea and the northern Baltic Proper differed only in their $\delta^{13}C$ values ($p < 0.001$).

Validation of food consumption showed a strong relationship between model estimates and experimentally determined consumption values ($r^2 = 0.966$, $p < 0.001$, $n = 9$). The model constant did not differ significantly from zero ($t_{[7]} = -1.367$, $p = 0.214$) and the model slope did not differ from unity ($t_{[7]} = 0.157$, $p > 0.1$). There was also a good relationship between the predicted and observed WHO_{total}-TEq estimates ($r^2 = 0.466$, $p < 0.001$, $n = 24$), although relative differences between these were higher (Fig. 5). Again, the constant was not significantly different from zero ($t_{[22]} = 0.979$, $p = 0.338$) and the model slope did not differ from unity ($t_{[22]} = 0.310$, $p > 0.1$). Mean congener-specific assimilation efficiencies predicted by the bioenergetics model were comparable with those observed by Isosaari et al. (2005). The variation between salmon individuals in predicted values was high but the mean values of most important congeners in WHO_{total}-TEq (high toxic equivalent factor value) were estimated well (Table 1). Further, there was good correlation ($r^2 = 0.652$, $p < 0.001$, $n = 29$) between assimilation efficiencies used in the model (AE_w) and *n*-octanol–water partition coefficients (log K_{ow}) (Table 1).

According to the model, a major part of the OCs in the Bothnian Sea and Gulf of Finland salmon originated from herring but accounted for only 17% in the northern Baltic Proper salmon, where the bulk of their OCs came from sprat (Table 2). In the Gulf of Finland, 13% of the total OCs in salmon originated from sprat. In the Bothnian Sea, sprat had the least impact on salmon OC concentration, whereas threespine stickleback was the second most important source, after herring, of WHO_{total}-TEq in Bothnian Sea salmon. According to food consumption (C, g·g^{-1}) estimates of the model, salmon in the Gulf of Finland consumed 20% more prey relative to other study areas.

Fig. 6. (*a*) Scatterplot of Baltic salmon (*Salmo salar*) $\delta^{15}N$ values in relation to salmon WHO_{total}-TEq concentration in the three study areas. The lines indicate the study area specific linear relationships. (*b*) Baltic Sea salmon trophic position (calculated from $\delta^{15}N$ values using cladocerans as baseline values) in relation to ANCOVA lipid-adjusted log WHO_{total}-TEq concentrations. The solid line indicates the fitted linear relationship and the broken lines represent the 95% confidence limits. Diamonds, Bothnian Sea; squares, northern Baltic Proper; circles, Gulf of Finland. Open symbols, 0+ seawinter salmon; solid symbols, 1+ seawinter salmon; shaded symbols, 2+ seawinter salmon.



The WHO_{total}-TEq concentration of Baltic salmon was strongly related to $\delta^{15}N$ in all study sites (Fig. 6*a*). Regression intercepts differed significantly between sites ($F_{[2]} = 15.913$, $p = 0.001$), but no difference was found in regression slopes ($F_{[2]} = 0.078$, $p = 0.926$). To make all of the individuals comparable, OC concentrations were lipid-adjusted and $\delta^{15}N$ values were converted to trophic position

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using site-specific baseline $\delta^{15}N$ values of the primary consumers (cladocerans) (Table 2). The lipid-adjusted log WHO_{toxEq}-TEq of Baltic salmon was very strongly related to trophic position ($r^2 = 0.793$, $p < 0.001$) (Fig. 6*b*). Trophic position varied from 4.5 to 6.1, and individuals from the Gulf of Finland had the highest mean trophic position and also the highest variation. Salmon from the northern Baltic Proper showed the lowest mean trophic position. Neither in analysis of all salmon individuals from three study areas together ($r^2 = 0.013$, $p = 0.594$) nor in analysis of individuals sampled only from the Gulf of Finland ($r^2 = 0.240$, $p = 0.151$) did we find any significant impact of time spent in the sea on trophic position. In data from the Gulf of Finland, all three sea-winter groups co-occurred.

Discussion

We found large differences in salmon WHO_{toxEq}-TEq concentrations between the three study sites in the Baltic Sea. Non-normalized mean OC concentrations of one-seawinter salmon in the Gulf of Finland were on average 5.6 pg·g^{-1} (33%) higher than the mean OC concentrations in salmon in the other areas. This mean difference increased to 8.5 pg·g^{-1} (48%) after lipid normalization, as salmon lipid content was considerably lower in the Gulf of Finland than in the other areas. These results differ from earlier observations (Isosaari et al. 2006) that non-normalized OC concentrations of 2-year-old Bothnian Sea salmon muscle were almost 9 pg·g^{-1} higher than concentrations in muscle of the Gulf of Finland salmon. This difference is probably due to the fact that the former salmon were collected during the spring spawning migration, which will lead to mixing of feeding populations, whereas our samples were all collected during the sea feeding phase. Baltic salmon spawning migration patterns vary significantly between different populations, and populations mix considerably in the feeding areas (Kallio-Nyberg and Ikonen 1992; Salminen et al. 1994; Salminen et al. 2001). Therefore, all sample collections should be carried out during the salmon feeding phase to allow valid conclusions about spatial differences in the accumulation of OCs from the diet. Moreover, our analyses were made from whole-fish homogenates, which can give different results compared with muscle tissue (Amrhein et al. 1999).

There was considerable variation in the bioenergetics model WHO_{toxEq}-TEq estimates compared with measured values. Within each study area, all salmon individuals were set to consume the same proportion of each prey species. In reality, there is substantial variation in prey species and size selectivity for individual salmon (Hansson et al. 2001). Stable isotope mixing models (Phillips 2001) could not provide quantitative information on the proportion of prey species in individual salmon diets because of the high overlap in C and N isotope values between prey species. However, comparing the trophic position of salmon in the isotope biplot relative to prey species reveals considerable differences in salmon diets between areas. After correction for trophic fractionation (here assumed to be 2.9‰ for $\delta^{15}N$ and 1‰ for $\delta^{13}C$), isotope ratios for salmon fall inside the area bounded by their prey species. According to mixing model theory, a predator's tissue isotope values reflect its diet (Phillips 2001). Intuitively, the shorter the distance is between predator and prey in an isotope biplot, the more this particular prey is consumed. Based on these tenets, salmon diet in the Bothnian Sea and Gulf of Finland mostly consisted of herring and threespine stickleback, whereas salmon diet in the northern Baltic Proper consisted mostly of sprat. These rough estimates are consistent with earlier stomach analyses (Andersson 1980; Hansson et al. 2001; Salminen et al. 2001). However, because of the limitations described earlier, the results concerning the composition of their diets must be viewed as tentative.

Diet proportions have a major effect on accumulation of OCs in salmon, as concentrations in the preferred herring size class were two to four times higher than in sprat. Highest OC concentrations in herring were observed in the Gulf of Finland where herring is the main salmon prey. Herring has suffered from retarded growth in the Gulf of Finland during the recent decade (Rönkkönen et al. 2004), which can lead to increased OC concentrations (Jackson 1996; Kiljunen et al. 2007; Peltonen et al. 2007). The contribution of each prey species in the salmon WHO_{toxEq}-TEq accumulation calculated by the bioenergetics model reflected these differences in the diet proportion and OC concentrations in prey. The bulk of the toxicants in the Baltic Proper salmon originate from sprat, whereas in the Bothnian Sea and Gulf of Finland, herring is the major source of contaminants in salmon.

Lower lipid content in prey fish might also have contributed to the higher OC concentrations in the Gulf of Finland salmon; according to the energy content analyses, the main prey species in the Gulf of Finland had up to 22% lower energy content per unit weight and almost 40% lower weight than herring of the same age at other sites. A low-energy diet, i.e., prey with lower lipid content, necessitates higher consumption to fulfil growth requirements and increase the intake of toxicants by salmon.

Variation in prey size was taken into account using a random prey size selectivity function (S. Hansson, Department of Systems Ecology, Stockholm University, SE-106 91 Stockholm, Sweden, personal communication (2004)). Such randomization of prey size is essential when predicting OC accumulation, as prey fish concentrations increase exponentially in relation to their length. If lengths of salmon prey are assumed to be normally distributed around the mean length, as in this study, an emphasis on larger prey will increase OC accumulation rates in the salmon. A prey fish picked randomly from the salmon "predation window" has a greater probability of yielding a high concentration because of the exponential increase in prey OC concentration in relation to its length. Hence, using only mean prey fish OC concentrations may lead to major underestimation of OC accumulation of salmon. Although a fully randomized model accounting for uncertainty of all parameters and input values in the model would be an optimal solution, such detailed information currently is not available. However, our results suggest that the uncertainty in OC accumulation of salmon is heavily related to the composition of salmon diets and that foraging preferences exhibited by individuals could explain a major part of the variation in the measured OC concentrations in salmon.

Within sites, we found a linear relationship between WHO_{toxEq}-TEq and $\delta^{15}N$ values, which also suggests that sal-

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mon OC accumulation is related to the composition of the salmon diet. Similar relationships between $\delta^{15}N$ and OC concentrations have been recorded previously, although intraspecific relationships have been found to be relatively weak (Kidd et al. 1995, 1998; Kiriluk et al. 1995). Weak relationships have been thought to result from different accumulation properties of ^{15}N relative to OCs, with other factors such as age, growth, and size being better predictors of OC accumulation (Berglund et al. 2001; Paterson et al. 2006). In contrast, we did not find these factors to be strongly related to non-normalized WHO_{total}-TEq concentrations in salmon; most of the variation was explained by $\delta^{15}N$ and lipid content. Slopes of the relationships between WHO_{total}-TEq and $\delta^{15}N$ were similar, suggesting that salmon bioaccumulate OCs to a similar degree at all study areas.

After using ANCOVA to lipid normalize the contaminant concentrations and combining the data from all study areas, trophic position of the salmon in the food web explained almost 80% of the observed variation in their concentrations of OCs. Site-specific variation in the OC concentrations seemed to relate to differences in trophic position. The highest and most variable trophic positions and the highest variation in the OC concentrations suggest higher dietary diversity within the salmon population in the Gulf of Finland. Some individuals are specialized to feed more on herring, which have a higher trophic position than the other prey species, whereas some individuals feed more on sprat or threespine stickleback and therefore have a lower trophic position. Variation could also arise from some salmon individuals selecting larger prey that have a higher trophic position. However, any prey size selectivity cannot be related to age or size of the salmon, as trophic position was not dependent on salmon length or time spent at sea.

The higher trophic position of salmon in the Gulf of Finland may have two explanations. Firstly, in the Gulf of Finland, salmon seem to feed mainly on prey items with a higher trophic position (herring). Secondly, stable isotope data suggest that prey species of salmon in general seem to occupy a slightly higher trophic position in the Gulf of Finland when compared with other areas. The slow growth of herring in the northern Baltic Proper and the Gulf of Finland during the past two decades is thought to be the result of a low number of large zooplankton prey (Flinkman et al. 1998; Rönkkönen et al. 2004). However, in the Gulf of Finland, high numbers of the nonindigenous predatory cladoceran *Cercopagis pengoi* have been observed in the herring diet since the 1990s (Peltonen et al. 2004). Although *C. pengoi* has also been observed in the Baltic Proper and the Bothnian Sea, its abundance has been lower than in the Gulf of Finland (Leppäkoski et al. 2002). *Cercopagis pengoi* has not been found in the diet of herring from the open sea of the Baltic Proper (Möllmann et al. 2004). *Cercopagis pengoi* has adapted well to the ecosystem in the Gulf of Finland, where its food niche overlaps with pelagic mysids, and therefore, it has a strong influence on the whole pelagic food web (Gorokhova et al. 2005; Lehtiniemi and Lindén 2006). *Cercopagis pengoi* is truly zooplanktivorous and obtains a higher trophic position than other zooplankters in the Gulf of Finland. Gorokhova et al. (2005) found a 0.8 shift in trophic level of young-of-the-year herring after invasion of *C. pengoi* in a bay along the Swedish coast. This is similar to the average difference (0.7) that we observed in salmon trophic levels between the Gulf of Finland and the other areas. Therefore, the high abundance of *C. pengoi* might partly explain the elevated trophic positions and increased OC concentrations in the salmon and its fish prey species in the Gulf of Finland.

This study has explored the trophic interactions and bioaccumulation of toxicants and investigated the methodological approaches. Knowing trophic interactions is essential, e.g., in ecosystem-based fisheries management, which has become increasingly important because of the increasing human impact on aquatic ecosystems. The current study indicates that average salmon OC concentrations in the Gulf of Finland are higher and vary more than the concentrations in the other areas in this study. Our results suggest that differences are due to (*i*) differences in the proportion of the three main prey species in the salmon diet between areas and individuals, (*ii*) spatial and species-specific variation in the OC concentrations in prey fish, (*iii*) spatial differences in prey fish lipid and energy content, and (*iv*) differences in the food web structure. Therefore, it seems that subbasin variations in the dynamics of prey species influence the OC concentrations in Baltic salmon, and the high OC concentrations in salmon might be decreased with suitable fisheries management of prey populations.

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Anguilla marmorata larval migration plasticity as revealed by otolith microstructural analysis

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Abstract: The oceanic early-life history of *Anguilla marmorata* was examined in the southwestern Indian Ocean in Mayotte, Mauritius, and Réunion islands through otolith microstructural analysis. The study of the hatching dates, the first feeding check diameter (FFD), the leptocephalus (LD) and metamorphosis (MD) durations, the age at recruitment (AR), and the leptocephalus otolith growth rate (OGR) of glass eels revealed great variations in early-life traits and relationships between them. An agglomerative nesting analysis discriminated three early-life histories, differently represented according to the locality: (*i*) fast migrants with short LD, short MD, young AR, large FFD, and high OGR dominated in Réunion and Mayotte; (*ii*) midspeed migrants with intermediate LD, MD, AR, FFD, and OGR dominated in Mauritius; (*iii*) slow migrants with long LD, long MD, old AR, small FFD, and low OGR were recorded only in Mauritius. All possible strategies were not observed and therefore not successful at the sampling time. However, several were simultaneously expressed, which suggests larval migration plasticity at the population level. This evidence is crucial information regarding both the species dispersal capabilities and the evolution from short-migratory tropical species towards long-migratory temperate ones in the genus *Anguilla*.

Résumé : L'histoire de vie larvaire d'*Anguilla marmorata* a été examinée dans le sud-ouest de l'Océan Indien à Mayotte, Maurice et La Réunion, par analyse de la microstructure des otolithes des civelles. Les traits de vie tels les dates d'éclosion, la marque de première prise de nourriture (FFD), les durées de vie leptocéphale (LD) et de métamorphose (MD), l'âge au recrutement (AR) et les taux de croissance de l'otolithe (OGR), se sont montrés variables et diversement corrélés. Trois types d'histoires de vie larvaire, discriminés par analyse de groupement agglomératif hiérarchique, ont été observés à l'échelle géographique : (*i*) migrateurs rapides à LD et MD courtes, AR jeunes, et FFD et OGR importants, dominants à La Réunion et à Mayotte; (*ii*) migrateurs intermédiaires à LD, MD, AR, FFD et OGR intermédiaires, dominants à Maurice; (*iii*) migrateurs lents à LD et MD longues, AR avancés, et FFD et OGR faibles, observés seulement à Maurice. Toutes les stratégies possibles n'ont pas été observées et ont donc été inefficaces pendent la période étudiée. L'expression simultanée de plusieurs stratégies laisse supposer, à l'échelle de la population, une plasticité de la migration larvaire chez *A. marmorata*. L'information est cruciale au regard des capacités de dispersion et du scénario d'évolution du genre *Anguilla* qui suppose une émergence des espèces tempérées aux migrations larvaires longues à partir des espèces tropicales aux migrations larvaires courtes.

Introduction

The duration of a fish larval stage, and therefore, its metamorphosis timing, might be key factors determining distances of dispersal and species distribution range (Arai et al. 2001*a*; Hoareau et al. 2007). Variation in oceanic migration duration of successfully recruited larvae probably reflects the species capabilities to delay metamorphosis and therefore the ability to maximize chances of finding suitable growth areas (Victor 1986). This delay has been observed to be very flexible in some fish groups and mostly for those composing the monophyletic group of the Elopomorpha

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Table 1. Duration (mean ± standard deviation, SD) of the leptocephalus stage of *Anguilla marmorata* reported in literature for glass eels sampled throughout the species distribution range from 1994 to 2005.

Locality	Year	*N*	Leptocephalus duration (days)	SD (days)	Min. (days)	Max. (days)	Range (days)	Source
Indonesia	1996	18	120	15.6	96	147	51	Arai et al. 1999
	1996	1	162	14.0	—	—	—	Marui et al. 2001
	1997	68	128	15.2	114	158	44	Arai et al. 2001*b*
	1999	23	79	11.2	—	—	—	Budimawan and Lecomte-Finiger 2005
Philippines	1994	10	120	13.0	105	140	35	Arai et al. 1999
	1998	16	146	19.5	112	183	71	Marui et al. 2001
Taiwan	1999	15	114	13.8	92	141	49	Arai et al. 2002
Japan	1999	15	123	13.9	100	155	55	Arai et al. 2002
Réunion	2001	9	97	26.4	60	135	75	Robinet et al. 2003
	2005	15	111	15.8	94	142	48	Present study
Mauritius	2005	30	139	24.0	91	180	89	Present study
Mayotte	2005	29	120	13.1	104	151	47	Present study

(i.e., Elopiformes, Albuliformes, Saccopharyngiformes, and Anguilliformes; Inoue et al. 2004).

In the complex catadromous anguillid eel life cycle, the duration of the leptocephalus stage, which undergoes an oceanic migration from spawning areas to growth coastal or inland waters, can vary widely among species. In eels, this larval phase is known to be shorter in tropical ancestral species than in the most recent temperate ones (Kuroki et al. 2006). Migration loops made by tropical species from intertropical spawning areas seem to have enlarged during evolution, offering the emergence of new species with large migration loops, with spawning areas still located in tropical waters while growth areas shifted toward temperate waters (Tsukamoto et al. 2002). This evolution could be consecutive to an intraspecific variability in the oceanic larval life duration, found in a number of recent studies, as for *Anguilla marmorata* (Marui et al. 2001; Arai et al. 2002; Robinet et al. 2003; Table 1), which is the most widespread species of the genus (Jubb 1961; Ishikawa et al. 2004; Robinet et al. 2007). This plasticity in the duration of the larval migration at the population scale might be the expression of selected strategies, which are defined here as genetically determined life histories or behaviours. Each individual will not show the whole strategy, but rather one of several tactics that the strategy may be composed of. The selection of strategies might occur according to environmental conditions (currents, water physico-chemical characteristics, trophic resource, etc.) and distance of the recruitment areas from the spawning location, maximizing the number of larvae that reach growth zones.

In the southwestern Indian Ocean (SWIO), *A. marmorata* spawning area was proposed to be unique and roughly localized northeast off the Mascarene Ridge by Jespersen (1942), Jubb (1961), and Robinet et al. (2008) (Fig. 1). In the present paper, we compare, through otolith microstructural analysis, the early-life traits (ELT: hatching dates, timing and duration of metamorphosis, age at recruitment, and otolith growth rate) of *A. marmorata* glass eels, sampled within the same month in three localities of the SWIO. On the one hand, Jespersen's hypothesis about a single regional spawning area is discussed in the light of the regional current circulation. On the other hand, variability of ELT is discussed in terms of response to variations of the pelagic environment and in terms of intra- and inter-specific dispersal capabilities.

Materials and methods

Study area and sampling

Réunion (21°S, 56°E; 2507 km^2) and Mauritius (20°S, 57°E; 1865 km^2) islands belong to the Mascarene Archipelago and are the most southern islands of the Mascarene Ridge (Fig. 1). Mayotte (12°S, 45°E, 374 km^2) is a part of the Comoros Archipelago located 1300 km northwest of the Mascarene Islands at the northern entry of the Mozambique Channel. These two archipelagos are bathed, respectively, by the southern and the northern bifurcation of the main regional current, the South Equatorial Current (SEC), flowing westward and splitting on the Mascarene Ridge and on the east coast of Madagascar (Schott and McCreary 2001).

74 glass eels and young elvers of *A. marmorata* were sampled in April 2005 in Mauritius (30 individuals), Réunion (15 individuals), and Mayotte (29 individuals) islands in the SWIO, with a portable electroshocker delivering electric impulses (DEKA 3000, EFKO manufacturer, DC 30 impulses·s^{-1}, 350 V, 4 A). Sampling occurred in the estuary of 26 permanent rivers as follow: eight rivers in Mauritius (5–7 April;, four in Réunion (11–12 April), and 14 in Mayotte (15–19 April).

Species identification

After fixation in 90% ethanol, eels were measured and identified using Ege's glass eel determination key (Ege 1939). Criteria used were caudal pigmentation and the distance between the origins of the dorsal and anal fins as a percentage of the fish total length: A–D (%) = [(LD – LA)/LT] × 100, where LD is dorsal length, LA is anal length, and LT is total length (Ege 1939; E. Réveillac, P.A. Gagnaire, R. Lecomte-Finiger, P. Berrebi, T. Robinet, P. Valade, and E. Feunteun, unpublished data). Identifications were confirmed through molecular analysis using semimultiplex PCR (Gagnaire et al. 2007). The pigmentation stage was determined according to Elie et al. (1982).

Otolith microstructural analysis

Otoliths can be regarded as a reliable record of early-life

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Fig. 1. Location of Mayotte, Mauritius, and Réunion islands in the southwestern Indian Ocean. The presumed spawning area of *Anguilla marmorata* by Jespersen (1942) is shown in grey; the Mascarene Ridge (thin dotted line) and main regional currents (solid and dotted lines with arrows) are also indicated: SEC, South Equatorial Current (dividing into the NEMC (Northeast Madagascar Current) and SEMC (Southeast Madagascar Current)).

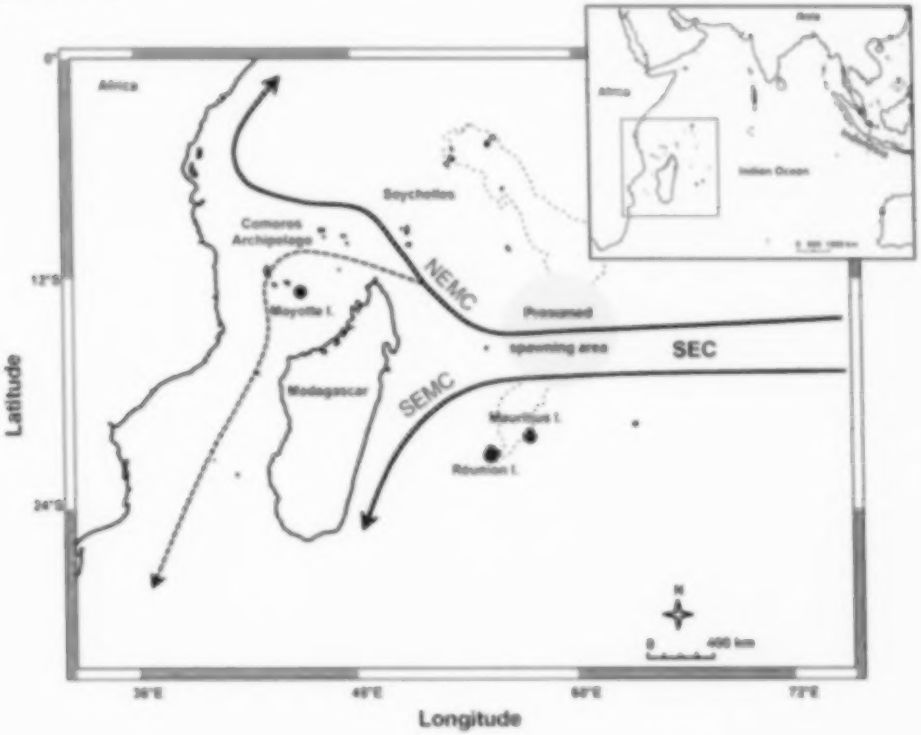
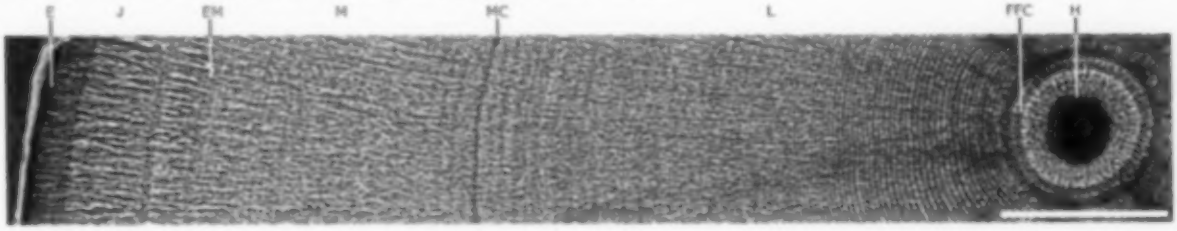


Fig. 2. Scanning electron microscope (SEM) photograph of the microstructure of an etched otolith of *Anguilla marmorata* with marks of developmental events. H, hatch check; FFC, first feeding check; L, leptocephalus stage; MC, metamorphosis check; M, metamorphosis; EM, end of metamorphosis; J, juvenile stage; E, edge of otolith. Scale bar = 20 µm.



history (ELH) (Lecomte-Finiger 1992; Campana 2001). Previous works on eel otolith microstructure revealed by scanning electron microscope (SEM) concluded that growth increment–deposition occurs daily with a low error margin (Martin 1995; Sugeha et al. 2001; Shinoda et al. 2004). Moreover, remarkable checks are thought to correspond to shifts between the different ontogenetic stages and, if increment–deposition is clear, enable the determination of the age of the individual at every shift (Wang and Tzeng 2000; Campana and Thorrold 2001; Jessop et al. 2006; Fig. 2). The otolith core can be observed as a deep hole in the centre of the etched otolith, with a surrounding ring described as the hatch check. The first feeding check, which is thought to correspond to the end of the preleptocephalus stage and to the complete absorption of the yolk reserve, is in the vicinity of the crystalline crown surrounding the core (Marui et al. 2001; Kuroki et al. 2005). The metamorphosis check, which marks the transition from the leptocephalus stage to the metamorphosis stage, is usually observed when increment widths start to increase suddenly (Lecomte-Finiger 1992). The end of metamorphosis, and therefore the onset of the glass eel stage, is represented by one or two successive annuli from which increment widths start to decrease towards the otolith edge (Lecomte-Finiger 1992). A total of 74 left sagittae of *A. marmorata* (30 from Mauritius, 15 from Réunion, and 29 from Mayotte) were extracted under binocular microscope, cleaned, embedded in metacrylate resin, and ground with 150, 9, and 3 µm

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grit paper until the nucleus was visible. Once polished, etched with 9% EDTA solution, and coated with gold, they were examined with an SEM (JEOL JSM 5410 LV). The age at first feeding could not be precisely determined because layers deposited within the crystalline crown surrounding the otolith core are not always well visible. Therefore, to characterize the preleptocephalus stage, we measured the diameter of the first feeding check (FFD in µm). This parameter and the duration of the leptocephalus (LD) and the metamorphosis (MD) stages, the larval otolith growth rates during the leptocephalus stage (OGR), the age at recruitment (AR) and, by back-calculation, the hatching date (HD) were determined for each individual. Otolith average growth rates (µm·day^{-1}) were calculated by the average thickness of every 10 increments from the FFD to the margin of each otolith, along the measurement axis.

Statistical analysis

Agglomerative nesting ("agnes"; hierarchical clustering), developed for R (R Development Core Team 2007; Kaufman and Rousseeuw 1990), was performed on the regional sample for FFD, LD, OGR, MD, and AR variables. The Euclidean metric system and the average method of linkage were used after the variables were standardized. A bootstrap analysis was performed to test the strength of the structure. An analysis of variance (ANOVA) was performed to test the differences for each variable between groups computed by the cluster analysis. The shape of the LD frequencies distribution was tested with MCLUST density test analysis of data aggregation (MCLUST: model-based clustering – normal mixture modeling) (Fraley and Raftery 2003, 2007), developed for R (R Development Core Team 2007). Means (± standard deviation, SD) of each variable were calculated for groups of individuals computed by the cluster analysis. Comparisons were made after performing Anderson–Darling normality tests and verifying variance equality with Student's t test or Kruskal–Wallis (KW) test. Correlation tests between variables were performed with the Spearman test for nonparametric data.

Results

Pigmentation and size at recruitment

The pigmentation of the 30 individuals caught in Mauritius was only developed on the caudal and rostral regions of the body (stages V_A to VI_{A3}), classifying them all as glass eels (Elie et al. 1982). Among the 15 individuals sampled in Réunion, four eels were at a more advanced pigmentation stage (VI_{A1} to VI_B), and one was classified as a stage VII (elver). In Mayotte, 14 eels were classified as stages VI_{A1} to VI_B.

Mean body length of all eels (i.e., all stages included) and only glass eels (V_A to VI_{A3}) were, respectively, 67.53 ± 32.97 mm (range: 50–178 mm) and 52.40 ± 1.07 mm (range: 50–54 mm) in Réunion Island and 59.76 ± 8.86 mm (range: 47–79 mm) and 52.53 ± 3.74 mm (range: 47–55 mm) in Mayotte Island. Mean body length of glass eels caught in Mauritius was 52.13 ± 2.60 mm (range: 46–56 mm). No significant variability in the size of glass eels occurred between the sites (KW test, $p > 0.05$).

Oceanic life

Anguilla marmorata glass eels sampled simultaneously in April 2005 showed a wide distribution range of HD from late August to late December 2004 (Fig. 3). The glass eels from Mauritius hatched significantly earlier (hatching date: 8 October 2004 ± 26.70 days) than those of Réunion and Mayotte, which showed quite similar distributions and mean HD (5 November 2004 ± 36.21 days and 6 November 2004 ± 23.11 days, respectively; KW test, $p > 0.05$).

The other ELT showed wide ranges of values at insular and regional scales (Fig. 3). They also exhibited interrelationships (Fig. 4), with LD being negatively correlated to FFD (Spearman, $R^2 = 0.746$, $p < 0.0001$) and to OGR (Spearman, $R^2 = 0.142$, $p < 0.01$) and positively correlated to MD (Spearman, $R^2 = 0.284$, $p < 0.0001$) and to AR (Spearman, $R^2 = 0.982$, $p < 0.0001$).

Three main clusters of individuals were computed by the agnes hierarchical cluster analysis (agglomerative coefficient = 0.88, bootstrap values > 98%; Fig. 5). They showed statistically different ELT values (ANOVA, $p < 0.05$) and corresponded to three modes of LD distribution frequencies observed at the regional scale (MCLUST density test, $E = 3$). The first group was composed of 30 individuals sampled in all three islands ($N = 10$ in Réunion, $N = 4$ in Mauritius, and $N = 16$ in Mayotte). Their life history was characterized by large FFD (mean 20.4 ± 0.5 µm), short LD (mean 104.6 ± 6.2 days, range 91–116 days), high OGR (mean 0.64 ± 0.08 µm·day^{-1}), short MD (mean 22.9 ± 2.5 days), and young AR (mean 127.5 ± 5.7 days). They were qualified as fast migrants. The second group (midspeed migrants) included 34 individuals also sampled in the three islands ($N = 5$ in Réunion, $N = 16$ in Mauritius, and $N = 13$ in Mayotte). They had intermediate values of FFD (mean 19.3 ± 0.4 µm), LD (mean 129.4 ± 10.3 days, range 117–151 days), OGR (mean 0.59 ± 0.06 µm·day^{-1}), MD (mean 26.7 ± 2.8 days), and AR (mean 156.1 ± 11.8 days). The third group (slow migrants) was composed of 10 individuals only sampled in Mauritius. They had small FFD (mean 18.1 ± 0.8 µm), long LD (mean 166.8 ± 47.6 days, range 159–180 days), low OGR (mean 0.55 ± 0.05 µm·day^{-1}), long MD (mean 29.3 ± 3.5 days), and old AR (mean 196.1 ± 8.0 days).

Geographical differences were observed, as each type of ELT combinations (i.e., ELH) was not similarly represented among islands. Indeed, fast migrants were dominant in Réunion and Mayotte, while midspeed migrants were the most represented in Mauritius recruitment. Slow migrants only occurred in Mauritius, where they were more abundant than fast migrants. The three types of ELH were therefore different in terms of ELT values and in terms of geographical occurrence.

OGR pattern

The OGR pattern from the centre to the edge of the otolith is shown (Fig. 6). It comprised four phases as described for *Anguilla japonica* (Arai et al. 1997); *Anguilla rostrata* (Arai et al. 2000); *Anguilla australis*, *Anguilla dieffenbachii*, and *Anguilla bicolor pacifica* (Marui et al. 2001); *Anguilla celebesensis*, *A. marmorata*, and *Anguilla bicolor bicolor* (Arai et al. 1999); and *Anguilla anguilla* (Lecomte-Finiger 1992). High individual variations were re-

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Fig. 3. Hatching date (*a*), first feeding check diameter (FFD, µm) (*b*), leptocephalus duration (days) (*c*), and metamorphosis duration (days) (*d*) frequencies distribution, determined by otolith microstructural analysis of 74 glass eels (*Anguilla marmorata*) sampled in the southwestern Indian Ocean in Réunion (N = 15), Mauritius (N = 30), and Mayotte (N = 29) islands in April 2005.

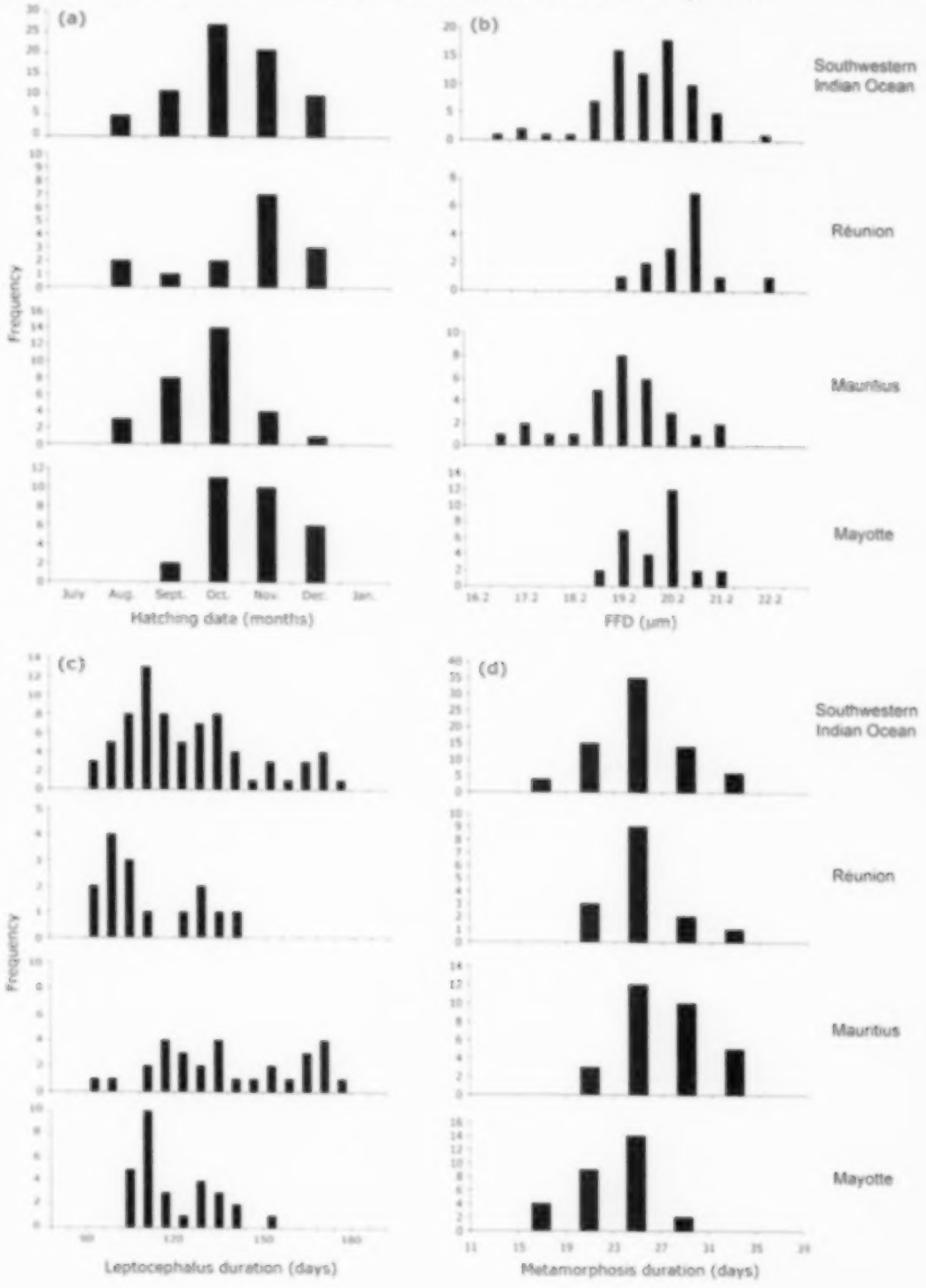

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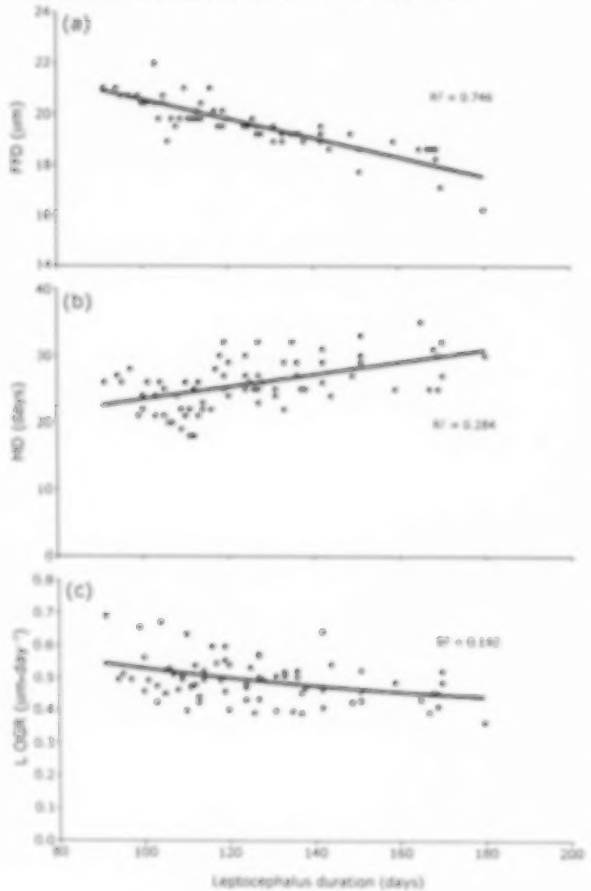
Fig. 4. Relationship between the first feed check diameter (FFD, μm) (*a*), the metamorphosis duration (days) (*b*), the leptocephalus daily otolith growth rate (L OGR) (μm·day^{-1}) (*c*), and the leptocephalus duration (days) in glass eels (*Anguilla marmorata*) caught in the southwestern Indian Ocean (N = 74) in April 2005.



corded within each phase, independently from the general pattern (Fig. 6). During the first phase, otolith increment widths increased between the hatch check and age 20–40 days (maximum peak average 0.94 ± 0.15 μm·day^{-1}, range: 0.68–1.30 μm·day^{-1}) and then gradually decreased until almost constant during the second growth phase (mean values 0.49 ± 0.08 μm·day^{-1}, range: 0.29–0.80 μm·day^{-1}). At the end of the steady growth phase, a sudden increase of the OGR occurred, corresponding to the third phase (maximum peak average 1.73 ± 0.35 μm·day^{-1}, range: 1.08–2.99 μm·day^{-1}). Increment widths then decreased afterward (fourth growth phase).

Discussion

Size at recruitment

In the present study, the body size at recruitment of *A. marmorata* (i.e., for juveniles at stages V_A to VI_{A0}) was poorly correlated to the migration duration, indicating no clear correspondence between these parameters at the intra-specific level. Nevertheless, mean sizes observed in each island (52.13 ± 2.60 mm in Mauritius, 52.40 ± 1.07 mm in Réunion, and 52.53 ± 3.74 mm in Mayotte) were close to those previously recorded for the same species recruiting into Indonesia (Arai et al. 1999, 2001*b*), into Taiwanese and Japanese coastal waters (Arai et al. 2002), and into Réunion estuaries (Robinet et al. 2003). Also, as observed in previous studies, these mean values were smaller than for temperate species at the same pigmentation stages, such as *A. anguilla* (68 mm) (Lecomte-Finiger 1992), *A. japonica* (57 mm) (Arai et al. 1997), *A. rostrata* (58 mm) (Arai et al. 2000), *A. dieffenbachii* (64 mm) (Marui et al. 2001), and *A. australis* (59 mm) (Shiao et al. 2001). Therefore, there is an obvious difference in length at recruitment between temperate and tropical species.

As temperate eels undergo much longer migrations than tropical ones (Lecomte-Finiger 1992, 1994; Kuroki et al. 2006), the size at recruitment seems, at the interspecific level, to be influenced by the LD, as suggested by Marui et al. (2001).

Clues for a single, regional spawning area

In the years 1928–1930, leptocephalus larvae were sampled in the Indian Ocean during the Carlsberg Foundation's Oceanographic Expedition Around the World. The results enabled Jespersen (1942) to locate a regional spawning area for anguillid eels at the northeast of Madagascar on the Mascarene Ridge (discussed by Robinet et al. 2008). However, further studies should be conducted to test Jespersen's hypothesis. Indeed, a model combining life history traits and oceanic circulation would help to simulate larval transport under various conditions. The global model NEMO (simulation DRAKKAR ORCA025-G70 over years 1959 to 2004, resolution 1/4 ; Penduff et al. 2007) computed 45 years of currents speeds and directions in the SWIO (Fig. 7). This model, roughly combined with migration durations we recorded in this study, seems to support the spawning area proposed by Jespersen (1942) and supported by Robinet et al. (2008). From this area, indeed, the SEC and the North Equatorial Madagascar Current could have transported larvae to Mayotte at the speed of about 20 cm·s^{-1} (Fig. 7). In that case, larvae would have crossed 1500 km in about 90 days, which roughly corresponds to the LD recorded for larvae recruited in this island. As well, from the Mascarene Ridge, the SEC and the South-Equatorial Madagascar Current could have carried larvae first to Mauritius and then to Réunion at the speed of about 10 cm·s^{-1}. Thus, those larvae would have crossed 1000 km in about 110 days, which is also consistent with the mean LD registered in those two islands.

Variability of ELH traits

Developmental variability was clearly expressed through obvious variations of early-life stages durations. The sum of these durations resulted in variations of ages at recruitment. This variability seemed to be also related to fluctuations of the OGR, which reflects individual somatic metabolic rates (Wright 1991). Relationship between developmental stages duration and OGR has already been described in other anguilliform larvae such as Anguillidae (Tzeng 1990; Robinet et al. 2008) and the American conger eel *Conger oceanicus*

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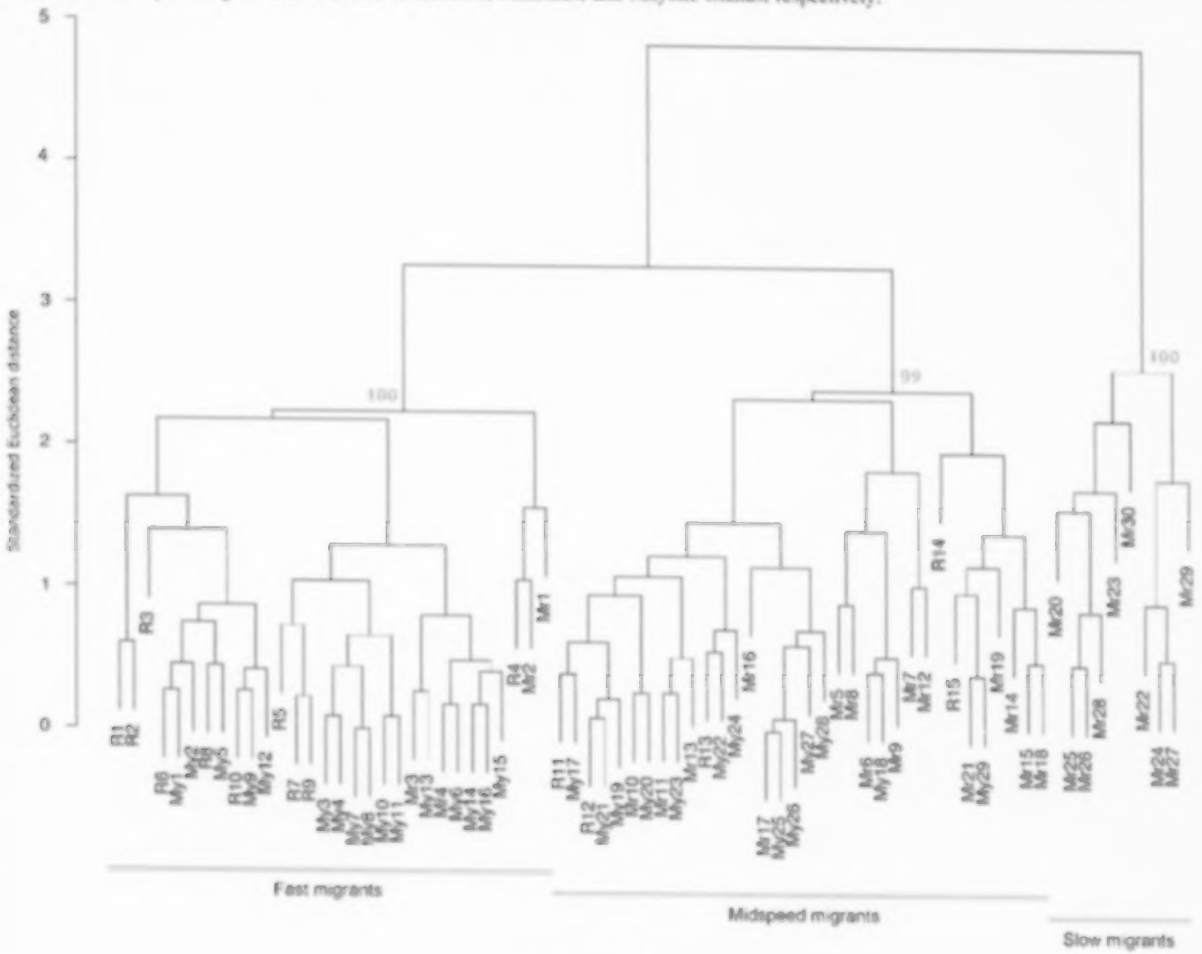
Fig. 5. Dendrogram computed by agglomerative nesting analysis (agnes; hierarchical clustering) of 74 individual glass eels (*Anguilla marmorata*) characterized by five early-life traits: first feed check diameter, leptocephalus duration, metamorphosis duration, otolith growth rate, and age at recruitment. Variables were standardized before the analysis of Euclidean distances. Bootstrap values are reported for the three identified strategies of larval migration: fast, midspeed, and slow migrants. R plus sample number, Mr plus sample number, and My plus sample number represent glass eels collected in Réunion, Mauritius, and Mayotte islands, respectively.



(Correia et al. 2004), but also in Pleuronectiformes with *Limanda ferruginea* (synonym *Pleuronectes ferrugineus*, in Benoit and Pepin 1999) or in some reef fishes (Wellington and Victor 1992). This study goes further, demonstrating correlations between more than two ELTs. These complex relationships discriminated individuals into three groups and revealed the existence of at least three ELHs (Fig. 8).

Observed ELT variations may be explained by two alternative or complementary hypotheses: (*i*) the intrinsic metabolism hypothesis and (*ii*) the environmental conditions hypothesis. Indeed, both may govern modalities of energy expense and may have repercussions on ELT and then on ELH. Environmental conditions such as water temperature (Hoie et al. 1999; Otterlei et al. 2002; Bang et al. 2006) and food availability (Takasuka and Aoki 2006) are thought to be the primary determinant of larval growth rate and subsequent larval duration (Anderson 1988; Jenkins and King 2006). Then, their variations are thought to have mainly produced the different ELH recorded. As the HD was not related to the other ELT, observations of several different ELHs might not be related to temporal heterogeneity, but rather to spatial heterogeneity of environmental conditions. As the hypothesis of a single spawning area seemed plausible, it is likely that heterogeneous conditions in this spawning area and (or) several migration routes have produced the different ELH observed. The global NEMO model showed that migrations were possible within the mean durations recorded through otolith microstructural analysis. However, complexity of currents was undervalued because this model calculated the mean circulation over several decades. Over shorter periods of time, like a season, those currents are indeed reported to have more complex trajectories (Schott

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Fig. 6. Otolith increment widths along the preleptocephalus, leptocephalus, and metamorphosis stages of 74 glass eels (*Anguilla marmorata*) sampled in the southwestern Indian Ocean in April 2005.

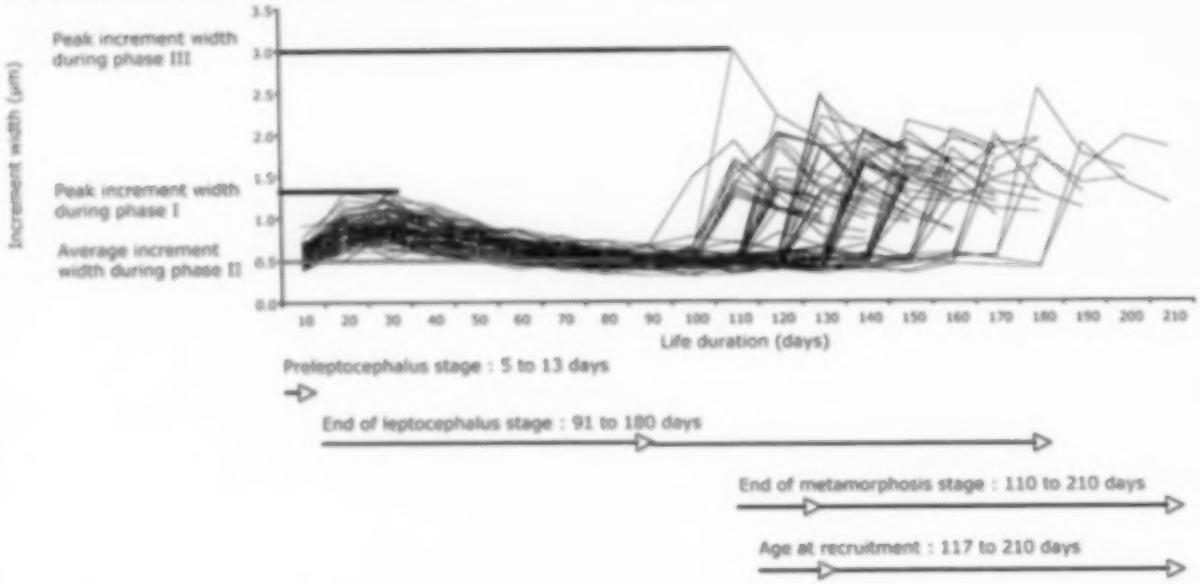
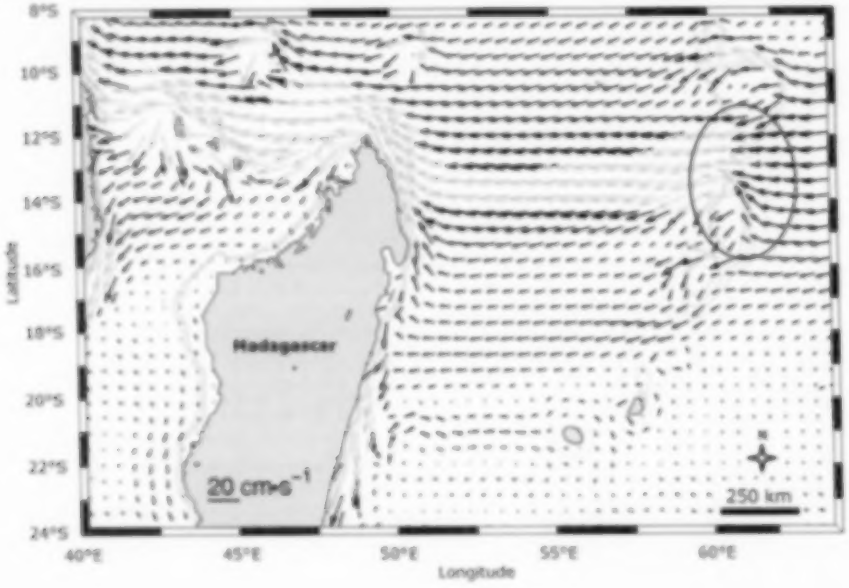

Peak increment width during phase III
Peak increment width during phase I
Average increment width during phase II
Increment width (µm)
Life duration (days)
Preleptocephalus stage : 5 to 13 days
End of leptocephalus stage : 91 to 180 days
End of metamorphosis stage : 110 to 210 days
Age at recruitment : 117 to 210 days

Fig. 7. Southwestern Indian Ocean mean currents speeds and directions (0 to 100 m depth) computed over years 1959 to 2004 by the global oceanographic model NEMO (simulation DRAKKAR ORCA025-G70, resolution 1/4°; Penduff et al. 2007). The spawning area of *Anguilla marmorata* proposed by Jespersen (1942) is delimited by a black ellipse on the Mascarene Ridge. Grey-shaded current vectors correspond to current velocities higher than 20 cm·s^{-1}.



and McCreary 2001; S. Pous, Université Pierre et Marie Curie, LOCEAN/IPSL, 4 place Jussieu, F-75252 Paris, CEDEX 5, France, unpublished data). As they encounter islands or shallow waters like the Mascarene Ridge, gyres are created (Padfield and Coward 1998). The turbulence of this system could partly explain how different migration routes could have transported larvae to different localities in different environmental conditions, inducing disparities in ages at recruitment and delays of arrival between localities.

In light of these results, more precise modeling is required to test hypotheses on *A. marmorata* migration routes in the SWIO. Active swimming, diffusion mechanisms, larval mor-

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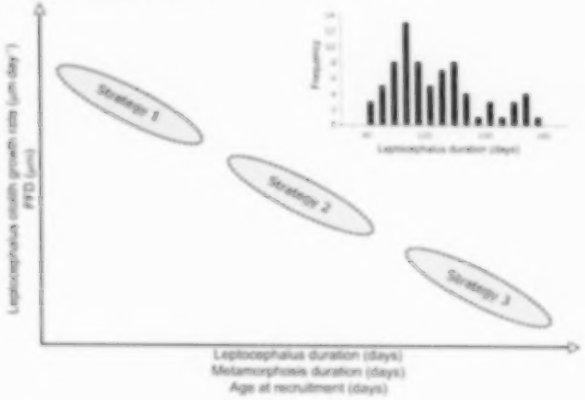
Fig. 8. Schematic representation of the three strategies (1, fast; 2, midspeed; and 3, slow migrants) discriminated by cluster analysis based on early-life trait combinations of glass eels (*Anguilla marmorata*) caught in April 2005 in the southwestern Indian Ocean. Frequencies of each strategy occurrence are shown in the top right corner. Other possible strategies were not observed. FFD, first feeding check diameter.



tality, and water temperature are some of the parameters that would have to be introduced in the model. In parallel, growth dynamic, which seemed to influence and (or) be influenced by the developmental rhythm at every stage of the oceanic life, has to be further examined to analyze the contribution of environmental and intrinsic factors to the anguillid eel larval life course.

A plasticity of larval migration in the anguillid eel?

As previously mentioned, variability of migration traits led to the observation of three ELH patterns, which respond to the following trend: the migration duration is inversely proportional to OGR and the associated metabolic rate. This variability demonstrates that *A. marmorata* larval migration can be achieved at various times. This might be a key in the migratory anguillid eel life cycle success. Indeed, several ELH patterns, qualified here as strategies, were expressed in variable proportions according to the recruitment place, while other strategies were not expressed. There is a possible trade-off between these various strategies. On the one hand, fast migration (linked to high metabolic rate) probably reduces mortality (Takasuka and Aoki 2006) while it also reduces the probability to find growth habitats. On the other hand, slow migration (low metabolic rate) might reduce larval survival while it might increase the probability to find suitable growth habitats. The existence of such a range of larval migratory strategies might globally increase the fitness of the population. It might also represent a strong selective advantage for *A. marmorata*, because it sustains an extraordinary dispersion capacity among fish species. Indeed, a short duration of the leptocephalus stage might favour geographical retention, while a long migration duration might favour both emigration (Correia et al. 2004) and connectivity between populations separated during their growth phase in continental systems.

The fact that no other strategies were observed could reflect unsuccessful or inexistent combinations of ELT. As McCormick (1999) proposed, larval duration of a fish is a product of its genotype, its larval environment, and the capacity of the species to delay metamorphosis. The lower age limit for settlement is governed by the rate of larval development, while the upper age limit is determined by the extent to which a delay of metamorphosis is possible, both also linked to the timing of finding suitable places to settle. On the one hand, short migration with low growth rate might be lethal because larvae that reach rapidly coastal shelves are not ready to undergo metamorphosis (i.e., are not competent for recruitment; Victor 1986; Hickford and Schiel 2003). On the other hand, long migration with high growth rate might induce rapid consumption of energy reserves, leading to body depletion and therefore death before coastal waters are reached.

Regarding the evolution of the genus *Anguilla*, short migration with high metabolic rate could be seen as the ancestrally successful strategy in tropical waters wherein the genus seems to have originated (Aoyama et al. 2001; Lin et al. 2001). Long migration with low growth rate could then be regarded as the strategy that has generated large migration loops leading to the emergence and establishment of temperate species. This strategy could have started to be profitable and widely represented when oceans opened, allowing larger dispersal toward temperate waters. This possibility of extending the dispersal range could have been supported by a lower temperature of migration, which, by decreasing the larval metabolic rate, led to viable migration strategies establishment (Gillooly et al. 2002; O'Connor et al. 2007). This scenario hypothesis must be examined in anguillid eels to deepen the knowledge on their evolution.

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Effect of changing land use patterns on the distribution of coho salmon (*Oncorhynchus kisutch*) in the Puget Sound region

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Abstract: Population increase in the Pacific Northwest of North America over the last century has led to the removal of forests for various purposes. Evidence of salmon response to these alterations in land use is rare owing to a scarcity of fish population data and a high degree of interannual variation in abundance. We examined the relationship between the spatial distribution of spawning coho salmon (*Oncorhynchus kisutch*) and changes in land use from 1984 through 2001 at 84 sites in four rivers draining into northern Puget Sound. Changes in land use over this period were determined from LandSat imagery, county zoning designations, and aerial photographs. Substantial reduction in forest cover occurred in many of the index watersheds during this time. The proportion of salmon using sites subjected to increased urban land use over the study period declined about 75%. Increases were observed at forested sites and those with increased rural residential use. Maintaining salmon populations in rapidly developing areas may require the identification and protection of sites that support large salmon populations and steering development to areas supporting few fish.

Résumé : La croissance démographique dans la région pacifique du nord-ouest de l'Amérique du Nord au cours du dernier siècle a entraîné l'élimination de forêts dans des buts divers. Des informations sur la réaction des saumons à ces modifications de l'utilisation des terres restent rares à cause de la pénurie de données démographiques sur les poissons et de l'importance considérable de la variation interannuelle de l'abondance. Nous examinons la relation entre la répartition spatiale des saumons coho (*Oncorhynchus kisutch*) durant la fraie et les changements d'utilisation des terres de 1984 à la fin de 2001 à 84 sites dans quatre rivières qui se déversent dans le nord de Puget Sound. Les changements d'utilisation des terres durant cette période ont pu être déterminés à partir d'imagerie LandSat, de l'établissement du zonage au niveau des comtés et de photographies aériennes. Il y a eu des réductions importantes de la couverture forestière dans plusieurs des bassins versants concernés durant cette période. La proportion de saumons exploitant les sites soumis à une utilisation urbaine accrue durant la période d'étude a diminué d'environ 75 %. Des augmentations ont été observées dans les sites forestiers et ceux dans lesquels les résidences rurales se sont multipliées. Pour maintenir les populations de saumons dans les régions en développement rapide, il faudra sans doute identifier et protéger les sites qui contiennent de fortes populations de saumons et réorienter le développement vers les régions qui contiennent peu de poissons.

[Traduit par la Rédaction]

Introduction

The human population has increased dramatically in the Pacific Northwest over the last several decades and is expected to continue to increase rapidly. The population in Washington, Oregon, and Idaho has increased from about 7.7 million in 1980 to over 10.6 million in 2000. The population in this region is projected to exceed 13.4 million by 2020 (US Census Bureau 2005). The increasing human population will continue to drive the conversion of forests to more developed environments to satisfy the residential, transportation, and other requirements of new residents.

The effect of human land use on stream habitat often has been implicated as a major factor in the decline of salmon in the Pacific Northwest (Nehlsen et al. 1991; National Research Council 1996; Bradford and Irvine 2000) and there is ample evidence that human activities impact aquatic habitat condition (Naiman 1992; Northcote and Hartman 2004). This information has been used to develop measures to reduce the impact of various human activities on aquatic systems (Booth and Jackson 1997; Paul and Meyer 2001; Ice 2004), and many millions of dollars have been spent restoring salmon habitat in the Pacific Northwest over the last decade (Bernhardt et al. 2005; Roni 2005). The underlying assumption to salmon habitat protection and restoration is that land use impacts can be mitigated sufficiently to enable the maintenance and (or) restoration of conditions suitable for native aquatic fauna, including salmon. In fact, most restoration plans implicitly assume that projects will be successful regardless of the land use context in which they are

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deployed (Independent Science Advisory Board 2007). The validity of this assumption is unknown, as the response of salmon populations to the implementation of protection or restoration measures has rarely been evaluated (Independent Science Advisory Board 2003; Bernhardt et al. 2005). A thorough understanding of the impact of land use change on salmon will be required if efforts to retain these fishes in the region are to be successful.

The lack of information on salmon response to land use regulations or restoration efforts stems in large part from the dearth of fish population data (Botkin et al. 2000). These types of data are difficult and expensive to obtain and accurate assessment of population-level responses would require data collected over a long period of time (Ham and Pearsons 2000). In cases where a long record of fish population data is available, such analyses have been possible (Kareiva et al. 2000; Paulsen and Fisher 2001). However, such data sets are rare and often not available for areas where human population growth, and consequent changes in land use patterns, have been most prevalent.

One of the few, consistently collected data sets for salmon in the Pacific Northwest are counts of spawning salmon. Generally, these data are thought to be inappropriate for assessing impacts of freshwater habitat condition on fish populations owing to the confounding influence of mortality in the marine environment (Bottom et al. 2006). However, if the effect of human activities on freshwater habitat is an important determinant of salmon population performance, some evidence of this fact should be reflected in the number of salmon returning to spawn.

Assessing the effect of land use on fish populations also requires accurate characterization of land use patterns. The ability to classify land cover and to identify changes in land use over time has been greatly facilitated over the last two decades by the rapid development of remote sensing tools and geographical information systems (GIS). Frequently, assessments of land use change have been accomplished by coupling satellite imagery with georeferenced databases of various types (Gould 2000; Lillesand and Kiefer 2000; Kerr and Ostrovsky 2003). Environmental changes have been evaluated using these techniques in a range of ecosystems (Mouat and Lancaster 1996; Zomer et al. 2001; Parmenter et al. 2003) including applications in remote areas where ground-based evaluations would be difficult or impossible (Making and Marsh 2001; Nagendra 2001; Franklin 2002).

To determine if changes in the distribution of salmon were related to changes in land use, we conducted a change detection using remote sensing and GIS data combined with surveys of spawning coho salmon (*Oncorhynchus kisutch*) collected over an 18-year period from 84 stream reaches in four large basins draining to northern Puget Sound. Coho salmon were selected for this analysis because this species has a lengthy period of freshwater residency (approximately 18 months) before migrating to the ocean and therefore should be more sensitive to alterations in freshwater habitat conditions than species that spend only a short period in freshwater.

Materials and methods

We assessed the changes in the distribution of spawning coho salmon in the Lake Washington, Snohomish River, Stilliguamish River, and Skagit River basins from 1984 through 2001. These four basins total nearly 22 000 km^2, extending from Puget Sound on the west to the crest of the Cascade Mountains on the east. The sites within these basins for which spawning salmon count data are available, termed "index reaches" (Fig. 1), are located in King, Snohomish, and Skagit counties. These counties have experienced explosive population growth and land development over the last several decades.

Counts of spawning coho salmon are conducted multiple times during the spawning season at the index reaches by the Washington Department of Fish and Wildlife (WDFW). These surveys are intended to provide an estimate of the abundance of fish produced by natural spawning. For this reason, index sites are not located near hatcheries or hatchery release sites. We utilized data only from those sites that had been surveyed at least three times each year for the years 1984–2001. Eighty-four index sites within the four study basins met this criterion. The index reaches used in the study averaged 1 km in length and ranged from 0.2 to 3.9 km. The index sites were all located on small channels. The watershed area above the index reaches was estimated using standard GIS tools and averaged 690 ha, ranging from about 10 to 2400 ha. Coho salmon prefer small streams for spawning (Groot and Margolis 1991). This behavior enables visual surveys of spawning fish, as seeing the salmon in these smaller streams is much easier than it would be on wide, deep channels. The cumulative area of all 84 watersheds above the index sites was approximately 570 km^2.

An estimate of salmon abundance at each index reach for each year was generated using the area-under-the-curve method (English et al. 1992). This estimator assumes a linear change in fish abundance between survey dates. The number of fish at a site on each day is summed over the spawning period to produce a statistic termed "annual fish days". This value provides an index of the number of salmon spawning at an index reach during each year. In cases where the first or last survey during a spawning season produced a count greater than zero, we assumed that the count was zero 1 week before or after. Fish days were calculated using the equation from English et al. (1992):

$$\mathrm{AFD} = \left[\sum_{n=1}^{x}(t_i - t_{i-1})(p_i + p_{i-1})\right]/2$$

where AFD is annual fish days, t is the length of time in days since the first survey of the year, p is the number of live coho salmon counted on each survey date, and x is the number of surveys. Fish abundance was then normalized for the length of each index reach (fish days per kilometre).

The normalized fish-day value for each index site was adjusted to account for differences in harvest rate among the four basins. This calculation was made using the following equation:

$$\mathrm{HAFD} = \mathrm{NAFD}/(1 - \mathrm{HR})$$

where HAFD is harvest-adjusted annual fish days corrected for harvest, HR is harvest rate for the year in which the surveys were conducted, and NAFD is annual fish days normalized for the length of each index reach.

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Fig. 1. Location of the study area (48°0′N, 122°30′W). Locations of the watersheds above the index reaches for which coho salmon (*Oncorhynchus kisutch*) data were available are indicated by the hatched polygons; data from 84 watersheds were included in the study. Many of the polygons contain multiple watersheds. Land use zoning designations across the study area are indicated by color.

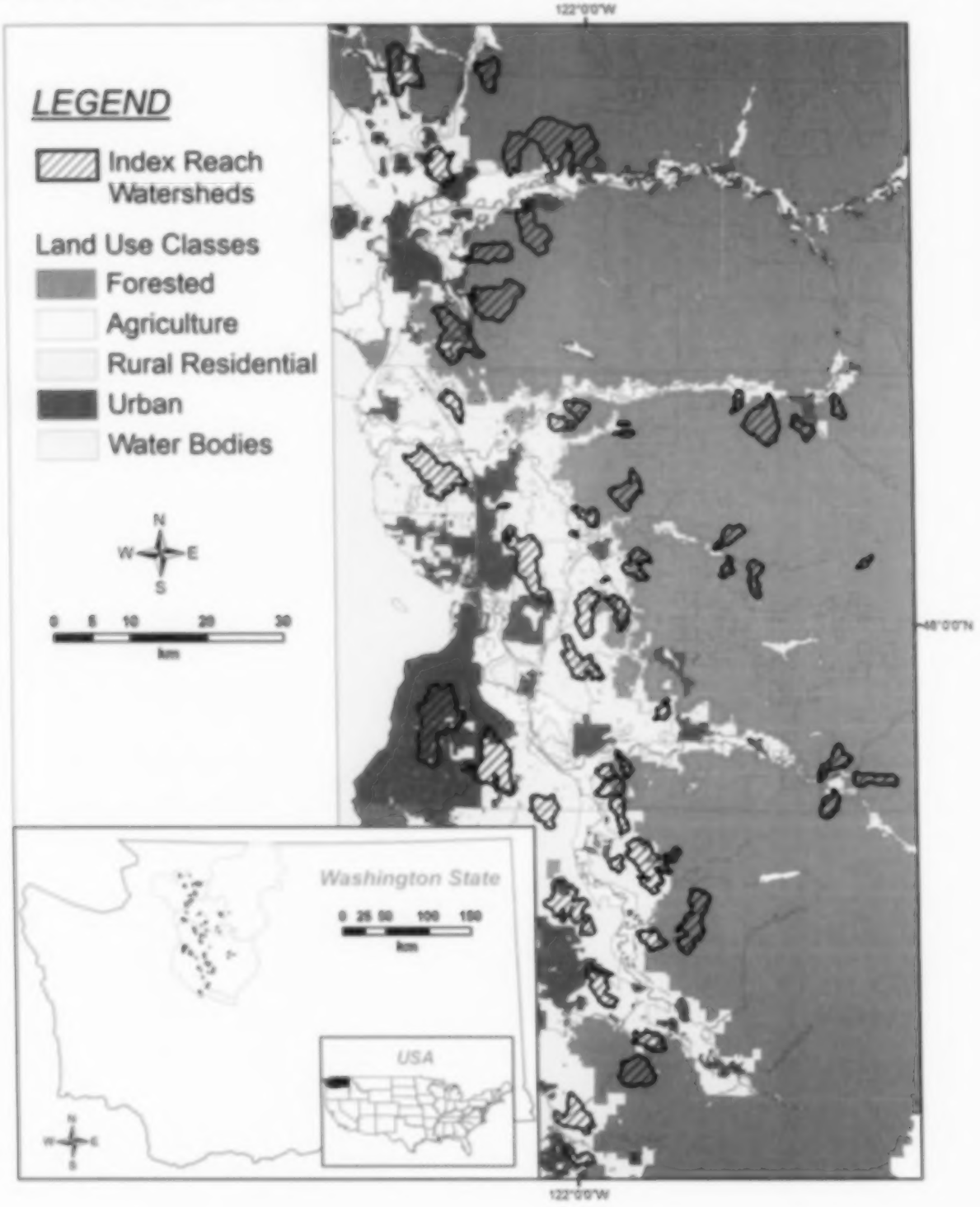

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Table 1. Source of the harvest rate data used to adjust annual fish-day estimates at the study sites.

	Source of harvest rate data		
Year	Skagit	Snohomish/Stilliguamish	Lake Washington
1984	Cascade River hatchery	Wallace River hatchery	Soos Creek hatchery
1985	Cascade River hatchery	Wallace River hatchery	University of Washington hatchery
1986	Cascade River hatchery	Wallace River hatchery	University of Washington hatchery
1987	Cascade River hatchery	Wallace River hatchery	University of Washington hatchery
1988	Cascade River hatchery	Wallace River hatchery	Soos Creek hatchery
1989	Baker River wild	Wallace River hatchery	University of Washington hatchery
1990	Baker River wild	Wallace River hatchery	Soos Creek hatchery
1991	Baker River wild	Wallace River hatchery	Soos Creek hatchery
1992	Baker River wild	Wallace River hatchery	Soos Creek hatchery
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1996	Baker River wild	Wallace River hatchery	Soos Creek hatchery
1997	Baker River wild	Wallace River hatchery	Soos Creek hatchery
1998	Cascade River hatchery	Wallace River hatchery	Puget Sound wild
1999	Cascade River hatchery	Wallace River hatchery	Puget Sound wild
2000	Baker River wild	Wallace River hatchery	Puget Sound wild
2001	Baker River wild	Wallace River hatchery	Puget Sound wild

Note: Data from wild populations were used when available and tagged hatchery fish when wild tags were not. In the case of Lake Washington, data from tagged hatchery fish were only available for four years (University of Washington hatchery). For other years, harvest rates from the Soos Creek hatchery, on the Green River, were used. As noted in the text, only fish without adipose fin clips (the traditional mark used to designate coded-wire-tagged salmon) were used to make the estimate after 1998, when mark-selective fisheries were initiated on these stocks. Unclipped, tagged fish were not released from the Soos Creek hatchery and average Puget Sound exploitation rates for wild coho salmon were applied to the Lake Washington estimates beginning in 1997. More detailed description of the derivation of these estimates may be found in the text.

Estimates of harvest rates for each year from 1984 through 2001 (Table 1) were provided by the WDFW (J. Haymes, WDFW, 600 Capitol Way North, Olympia, WA 98501, USA, personal communication) and based on coded-wire tag data (www.rmis.org). Coded-wire tag data for wild coho salmon were available for the Skagit River in some years. These data were used when available. If wild fish data were not available, data from tagged hatchery fish were used to represent harvest rate on wild fish. There were no coded-wire tag releases from the Stillaguamish River, so data from the Wallace River hatchery, on the Snohomish River, were used to approximate harvest rate for fish from both the Snohomish and Stillaguamish rivers. Tagged fish were released in the Lake Washington basin during only five years from 1984 to 2001. For years without Lake Washington releases, we utilized coded-wire tag data from the Soos Creek hatchery in the Green River basin, adjusted based on the ratio of harvest rates of the Lake Washington and Soos Creek fish for years when data from both basins were available. The Green River system is immediately south of the Lake Washington basin and both drain into Puget Sound in Seattle. A selective fishery for hatchery coho salmon began with the 1998 brood year and all hatchery fish were marked with an adipose clip, the traditional external mark used to denote a coded-wire-tagged fish. Therefore, coded-wire-tagged fish with an adipose clip were subjected to higher harvest rates than the unclipped, wild fish. To determine wild fish harvest and marine survival rates, unclipped, coded-wire-tagged fish also have been released from some hatcheries since 1998. Exploitation rates for these unclipped, tagged fish were used in making our harvest correction starting with the 1998 brood year. No unclipped, tagged fish were released from the Soos Creek hatchery and average wild coho salmon harvest rates for Puget Sound were used to estimate Lake Washington exploitation starting in 1998. The annual fish-day totals calculated from the fish counts at the index reaches were adjusted to account for harvest. These adjusted values represented an index of spawning salmon abundance that would have been achieved in the absence of harvest.

The harvest-adjusted annual fish-day values at each of the 84 index reaches for each year were converted to a proportion by dividing the fish days at each site by the sum of all harvest-adjusted fish days at all index sites:

$$\mathrm{PHAFD}_i = \mathrm{HAFD}_i / \sum_{n=1}^{84} \mathrm{HAFD}$$

where PHAFD_i is the proportion of harvest-adjusted fish days at index site i.

Expressing the fish data in this manner indicates how the spawning salmon were distributed among the 84 index sites each year. We chose to examine changes in spawner distribution because we believed that this metric would be more responsive to the impacts of changing freshwater habitat conditions than fish abundance as represented by the fish-day values. If the fish from these 84 index sites are being similarly affected in the marine environment, changes in the distribution of spawners among the index reaches over time should reflect changes in the relative quality of freshwater habitat among sites.

This approach does assume that the coho salmon from all

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the index sites experience similar, nonfishing mortality rates after entering salt water. These populations occupy the same marine areas, entering Puget Sound upon leaving freshwater and migrating through the Juan de Fuca Strait to the North Pacific Ocean. Marine mortality estimates based on coded-wire tags are available for the Skagit and Snohomish basins (www.rmis.org; tag data analyzed by J. Haymes, WDFW, 600 Capitol Way North, Olympia, WA 98501, USA) and these data support our assumption. Smolt-to-adult mortality rates owing to factors other than fishing ranged from 76% to 98% over our study period. Rates for the two basins were within 5% during 12 of the 18 years and mortality differed by more than 10% during only 2 years. Average difference in mortality rate between the two basins was 3.8% (SE = 0.9%).

Land use changes in the watersheds above the index sites between 1984 and 2001 were determined by conducting a change detection incorporating LandSat imagery, county zoning designations, and aerial photographs. LandSat images taken in 1986 (the earliest clear image of the entire study area that we could obtain) and 2001 were compared to identify areas from which forest cover had been removed. Zoning designations for the three counties in which the index sites were located were aggregated into four categories (Fig. 1). Urban included all industrial, commercial, and high-density residential development. Rural residential was assigned to all areas where housing density was restricted to less than one dwelling per 2.5 ha. All agricultural zone designations (e.g., pasture, row crops, and orchards) were grouped as agriculture. Areas included in our forest class included private land zoned for forestry, as well as state and federal forest land. We assumed that a loss in forest cover identified in the LandSat analysis represented a change to the land use designated by the zoning for that site. Loss of forest cover in an area zoned as forest was assumed to represent logging.

To assess the accuracy of the process that we used to assign land use changes, 10% of the locations where forest cover removal was identified in the LandSat images were validated against aerial photographs. Examination of aerial photographs indicated that our technique of assigning a land use change achieved an accuracy of about 94%. The most frequent error occurred in areas zoned for rural residential development where forest cover loss occasionally represented logging rather than construction of a dwelling.

To examine the relationship between change in coho salmon distribution and land use change, index watersheds were grouped according to the type of land use changes that they experienced during the study period. Watersheds included in the urban, agriculture, or rural residential land use change classes experienced conversion of forest to one of these land uses on >1% of the area above an index reach. Sites designated as forest experienced either logging or no change in forest cover over the study period. A forested site was considered to have experienced logging if >1% of the forest cover was removed by this activity between 1986 and 2001. The only sites where no loss of forest cover occurred between 1986 and 2001 were 12 watersheds that were zoned as forest where no logging occurred over the study period.

Typically, urban, rural residential, and logging impacted a much larger proportion of the watershed than the 1% threshold. However, conversion of forest to agriculture generally impacted only a small proportion of a watershed and most of the sites included in this land use change class experienced conversion of <5% of the watershed area to agriculture during the study period. Some index watersheds experienced more than one type of land use change over the period of the study. In these cases, index watersheds were included in both land use change groups. The proportion of harvest-adjusted fish days at each the index site in a land use change class for each year was summed. Trends over time in the proportion of harvest-adjusted fish days in each land use change class were examined to determine if land use change were related to shifts in the distribution of spawning coho salmon. Trends over time at individual index sites were also examined. Significance of the trends was evaluated using a Mann–Kendall trend test (Helsel and Hirsch 1992) and a significance level of $p \leq 0.05$. The Mann–Kendall test was chosen because no assumption of normality in the harvest-adjusted fish-day data was required.

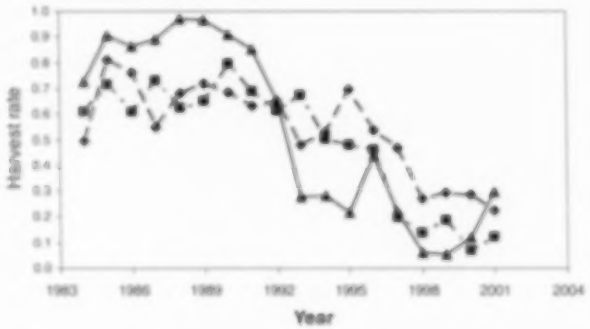

Fig. 2. Harvest rates on coho salmon (*Oncorhynchus kisutch*) for the four basins included in this study from 1984 through 2001. Rates are based on coded-wire tag data compiled and analyzed by J. Haymes (Washington Department of Fish and Wildlife). No tag data were available for the Stillaguamish River and rates for the Snohomish River are used to approximate harvest for this basin. Lake Washington harvest rates are partially based on coded-wire tag data from the Soos Creek hatchery on the Green River system. Diamonds, Skagit; squares, Stillaguamish/Snohomish; triangles, Lake Washington.

Results

Although there were differences among the four basins in the harvest rate of coho salmon during the study period, all exhibited similar patterns of change over time (Fig. 2). Harvest rates were high in all basins until about 1990, ranging from about 50% to over 90%. A sharp decline in harvest rate occurred in the mid-1990s as a result of increasingly restrictive fishing regulations in Washington and British Columbia. Rates since 1998 have not exceeded 30% in any of the basins.

Spawning salmon were unevenly distributed across the 84 index sites (Fig. 3) and there was considerable temporal variability in the proportion of harvest-adjusted fish days supported by a site. Some of the variation in the proportion of fish days supported by a site was related to changes in land use (see below). However, there was a tendency for the pro-

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Fig. 3. Average proportion of harvest-adjusted fish days at each of the 84 index reaches from 1984 through 2001. Error bars represent +1 SE.

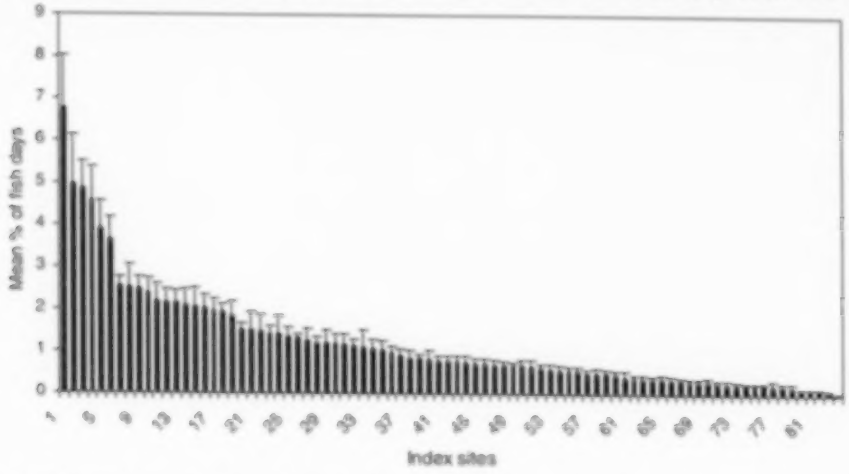

Table 2. Land use change between 1986 and 2001 in the watersheds above the coho salmon (*Oncorhynchus kisutch*) index stream reaches included in this study.

Land use change class	No. of index watersheds	Change in area from 1986 to 2001 (km^2)	Proportional change (%)
Agriculture	5	3.3	15.2
Urban/industrial	12	18.1	44.8
Rural residential	45	47.3	66.2
Forest	34	−68.7	−15.8

Note: Loss of forest cover was identified using LandSat imagery from 1986 and 2001. The land use change class resulting from the removal of forest cover was based on the county land use zoning designation for that location. See the text for details on the determination of land use change classes. Total watershed area above all 84 index sites was 5668.6 km^2.

portion of fish days each year to be concentrated at a relatively small number of index sites. For example, the 15 index sites with the highest average proportion of harvest-adjusted fish days supported more than 50% of the total. In contrast, many of the index sites supported relatively low proportions of harvest-adjusted fish days; the 42 index sites with the lowest average number of fish days accounted for only 17% of the total.

The LandSat imagery indicated that forest cover was removed from about 13 600 ha in the index watersheds between 1986 and 2001, 23.4% of the total area (Table 2). County zoning designations indicated that slightly more than half of this removal represented a change from forest to some other land use and the remainder was due to logging. In 1986, about 7% of the area of the index watersheds was urban. Agriculture occupied 4%, rural residential occupied 13%, and the rest was forested. By 2001, urban area increased to occupy nearly 11% of the index watershed area. Agriculture increased only slightly, to 4.4%, and rural residential development occupied 21% of the area. A total of 12 index sites included an increase in urban land use over the study period; the proportion of watershed area converted at these sites ranged from 1.5% to over 27% and averaged 14%. Only five index sites underwent an increase in agriculture, and generally, these increases were small, ranging from 1.6% to 3.8% of the watershed area, averaging 2.6%. Rural residential change influenced 45 watersheds, with loss in forest cover averaging 15% and ranging from 1.5% to 33%. Thirty-four index watersheds included areas designated as forest. Logging occurred in 22 of these watersheds, with loss of forest cover averaging 20% of the watershed area and ranging from 4% to 57%.

Index watersheds subjected to urban development during the study period exhibited a marked and continuous decline in the proportion of the population of spawning coho salmon that they supported (Fig. 4; Table 3). In the mid-1980s, these 12 index sites contained about 20% of the total spawning aggregation. By 2000, these sites supported <5% of the fish. The proportion of spawning coho salmon exhibited a statistically significant declining trend in eight of the 12 watersheds where urban development occurred. None of these watersheds exhibited an increase in the proportion of harvest-adjusted fish days supported. Three of the 12 watersheds with increased urban land use were among the top 20 index sites in terms of the proportion of spawning coho salmon that they supported in 1984–1987, and six were in the top 50%. By 1997–2001, the urbanizing watersheds included only one watershed in the top 20 and two in the top 50%.

Sites with increased agricultural land use exhibited a significant downward trend in the proportion of spawning coho salmon (Fig. 4; Table 3), although this trend was not nearly as steep as that noted for urbanizing sites. Of the five sites that exhibited an increase in agricultural land, three displayed a downward trend in harvest-adjusted fish days and two had no trend. Index watersheds with increases in rural residential development displayed a gradual increase in the proportion of spawning fish (Fig. 4; Table 3). Seventeen of the sites in areas with increased rural residential use did not

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Fig. 4. Change in the proportion of spawning coho salmon (*Oncorhynchus kisutch*) utilizing index reaches in the four land use change categories (*a*) urban, (*b*) agriculture, (*c*) rural residential, and (*d*) all forested sites) and in (*e*) forested sites where logging occurred between 1986 and 2001 and (*f*) forested sites where no logging occurred between 1986 and 2001. Note the change in the *y*-axis scale among panels.

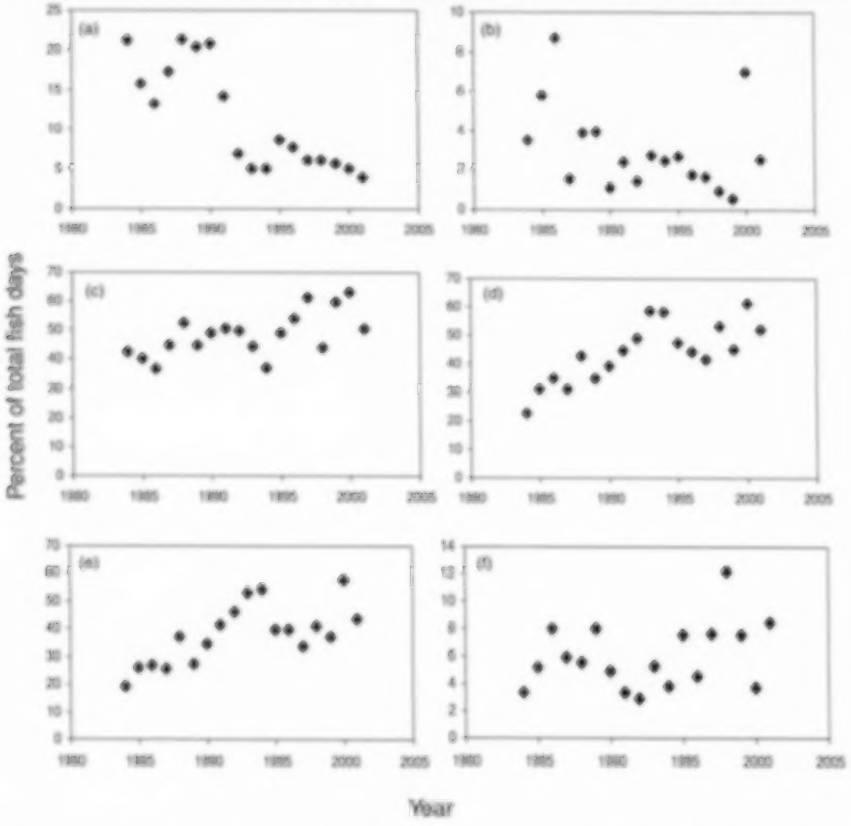

exhibit a trend; 22 of these sites trended upward and six downward. The forest sites exhibited the greatest increase in the proportion of spawning fish supported, from about 30% of the total spawning population at the start of the study period to about 55% at the end (Fig. 4; Table 3). Of these sites, 15 exhibited a positive trend, 13 no trend, and eight a negative trend. The subset of forested sites that experienced logging over the study period also displayed a significant increase in the proportion of fish days supported (Fig. 4; Table 3). Eleven of these sites experienced an upward trend in harvest-adjusted fish days, eight had no trends, and four had a downward trend. Those forested sites where no logging occurred exhibited no significant change in spawner utilization (Fig. 4; Table 3). Individual site responses at the forest sites with no logging were evenly divided, with four sites each with positive, negative, and no trends.

Table 3. Mann–Kendall trend test results for changes over time in the proportion of spawning coho salmon (*Oncorhynchus kisutch*) supported by each land use change category.

Land use change class	Trend direction	*p*
Urban	Down	0.000076
Agriculture	Down	0.047795
Rural residential	Up	0.007672
All forested sites	Up	0.000326
Forested, logging	Up	0.000948
Forested, no logging	Up	0.202335

Note: Values of $p \leq 0.05$ were assumed to represent significant evidence of a trend. The *p* value for upward trend is provided for the forested sites where no logging occurred, although the trend for these data was not significant.

Discussion

Land use changes in watersheds above coho salmon index reaches were associated with shifts in the distribution of spawning coho salmon between 1984 and 2001. The most striking change was a shift away from locations undergoing urbanization to watersheds less affected by development. There also was a marginally significant downward trend at index sites that experienced an increase in agricultural land use. There was a relatively small area converted from forest to agriculture in those watersheds where agriculture did increase and only six of the index watersheds actually exhibited an increase in this type of land use. As a result, it is unclear the extent to which these small increases in agricultural land use contributed to the downward trend in the proportion of spawning salmon at these sites.

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Increased proportions of spawning salmon were found at sites with increases in rural residential or forestry activity. These increases may not represent a true increase in salmon utilization. Because we examined proportional changes in spawning fish, any decrease in one land use change category had to be offset by an increase in another; the sum of all sites each year always totaled 100%. Therefore, increases at the forested and rural residential sites are primarily due to the rapid decline in the proportion of fish utilizing urbanizing watersheds. Increased carrying capacity at rural residential or forested sites seems unlikely to be a major factor in the increase in the proportion of spawning coho observed at these sites, although changes in the management of federal forest land and revision of regulations governing practices in state and private forests enacted through the 1980s and 1990s have elevated the level of protection afforded aquatic systems in forested watersheds (Forest Ecosystem Management Assessment Team 1993; Ice 2004).

The subset of forested sites where logging occurred during the period examined exhibited an increase in the proportion of spawning coho salmon; however, no significant trend was observed for forested watersheds where no logging occurred. Similarly, Bradford and Irvine (2000) also found that the proportion of a watershed that was recently logged was not correlated with declines in coho salmon abundance in the Thompson River, British Columbia. Two factors likely contributed to this seemingly anomalous result in our study. First, virtually all of the forested watersheds included in the study had been logged at some point in the past. Therefore, many of the impacts on aquatic habitat associated with logging (e.g., roads and modification of riparian vegetation) existed regardless of whether actual harvest occurred between 1984 and 2001. Second, the watersheds where no logging occurred supported a relatively small proportion of the spawning salmon. These 12 index sites accounted for between 3% and 12% of the spawning aggregation over the time period that we examined. In contrast, the 22 index sites where logging did occur supported from 30% to 60% of the spawning aggregation, increasing over time. The difference may be largely attributed to the fact that the sites without logging tended to be at higher elevation and exhibited greater stream gradient and confinement than those watersheds where logging did occur, conditions unfavorable to coho salmon (Burnett et al. 2007). The poorer habitat conditions and lower abundance of coho salmon may have made it more difficult to detect a trend at the sites where no logging occurred. Therefore, the difference in response between sites with and without logging likely is unrelated to whether or not trees were removed from the watershed during the study period.

It is unlikely that the patterns of change that we observed in coho salmon distribution related to land use changes are an artifact of inaccuracies in the data. Inconsistencies in the salmon count data can be caused by varying water level and clarity, variations in light level among survey dates, or changes in survey crews. Also, some of the index sites had relatively few surveys in some years (three to five), often requiring our assumption of linear change in spawner density between survey dates to extend over a period of several weeks. We also made assumptions about harvest rates that could have influenced our estimates of harvest-corrected fish abundance. We did not have complete coded-wire tag data for wild coho salmon for any of the basins, and complete hatchery tag data were only available for the Skagit and Snohomish basins. These deficiencies required the use of tag data from adjacent basins to develop harvest estimates for the Stillaguamish and Lake Washington basins. However, there is no reason to believe that any inaccuracies in the data would have been greater for sites in one land use change class than another. In addition, the magnitude of the change in coho salmon distribution with changing land use was large enough that even fairly substantial errors in our assumptions or estimates would not have fundamentally altered the outcome. In fact, we ran the analysis without correcting for differential harvest rates among the four basins and the same association between changes in fish distribution and changes in land use was still observed.

Impacts of urbanization on stream fish communities have been widely reported and typically occur at relatively low levels of development. Changes in fish communities were observed in streams in Wisconsin and Minnesota when connected impervious surface levels reached 6%, and trout were absent at values over 11% (Wang et al. 2003). Coho salmon seem to be particularly sensitive to urbanization. Coho salmon were virtually absent from a heavily urbanized stream near Seattle but were the dominant species in a nearby stream in a rural area (Scott et al. 1986). Evaluation of watersheds with varying levels of urban development revealed a sharp decline in the representation of coho salmon in a fish community with increasing levels of urbanization up to about 10% impervious area with a near total replacement of coho by cutthroat trout (*Oncorhynchus clarkii*) at levels above this (May et al. 1997). The work presented here corroborates these earlier results and also demonstrates that urbanization is associated with a decline in abundance of adult salmon.

Urbanization has a variety of effects on streams, many of which can impact fish communities. Runoff in urban areas with impervious surface levels of 10%–20% is double that in forested catchments and increases to more than fivefold at impervious surface levels over 75% (Arnold and Gibbons 1996). Levels of chemical contaminants, including metals, excess nutrients, pesticides, and toxic organic compounds, also are typically elevated in urban environments owing to road runoff, industrial and sewage effluents, and runoff from yards and many other sources (Paul and Meyer 2001).

Coho salmon may be more sensitive to urban development than some other fishes. This species spawns in the autumn and early winter in the Puget Sound region. Therefore, their eggs are incubating in the streambed gravel through the winter, when precipitation and stream flows are typically high. In the Seattle area, flows with an expected return interval of 10 years in a forested landscape occur every 2 years or less in urbanized watersheds, producing unstable bed conditions (Booth and Jackson 1997). As a result, salmon eggs are exposed to a high risk of being scoured from the gravel or being suffocated by deposited sediment. Impacts of elevated flows on incubating eggs are much reduced for species that spawn in the spring, such as cutthroat trout.

Very high rates of prespawn mortality of adult coho salmon have recently been documented in urban streams (N. Scholz, National Oceanic and Atmospheric Administra-

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tion, National Marine Fisheries Service, 2725 Montlake Boulevard East, Seattle, WA 98112, USA, unpublished data). Mortality rates in several urban streams in the Seattle area have ranged from 24% to 86% in recent years. In contrast, prespawn mortality rates in streams in forested areas over that time were about 1%. High rates of prespawn mortality coupled with the elevated risk of egg mortality owing to altered hydrology and sediment transport could easily produce the rapid decline in coho salmon utilization of spawning habitat in urbanizing areas that we observed.

The human population in the area where we conducted this analysis (King, Snohomish, and Skagit counties) increased from about 1.7 million in 1980 to over 2.4 million by 2000 (Office of Financial Management 2002). This population increase generated substantial conversion of forest to other land uses in the watersheds above the index reaches. Relatively little land was converted from forest to agriculture. Most of the conversion that occurred over this time period was due to urban and rural residential development to accommodate the large increase in population. By 2025, the population in these three counties is expected to expand to between 2.8 million and 3.6 million. As a result, land will continue to be developed to meet the residential, commercial, and transportation needs of the growing population.

The impact of this new development on coho salmon will depend on where it occurs. Currently, nearly 70% of the total land area in the four basins where we conducted our study is zoned or otherwise designated as forest land. However, much of this forest land is above barriers to salmon migration. The areas occupied by coho salmon tend to be at lower elevations and in areas of relatively low topographic relief. These also are the locations most suitable for development or agriculture, as evidenced by the widespread conversion of floodplain forests to agriculture throughout the Puget Sound region (Collins et al. 2003). Current zoning directs most future development to areas below barriers to anadromous fishes, often along major river corridors. Only 35% of the lands adjacent to stream reaches that are accessible to coho salmon in the four basins where we conducted this study are designated as forest and another 35% are zoned for rural residential development. About 18% of the land adjacent to salmon-accessible streams is zoned for agricultural land use and 12% is designated for high-density residential, commercial, industrial, or other urban uses.

We found that the abundance of spawning salmon was not evenly distributed among the index sites that we examined. Other studies have also noted this uneven spatial distribution of salmon and have attributed this pattern of distribution to variations in watershed and stream channel characteristics (Pess et al. 2002; Feist et al. 2003). This patchy distribution of salmon abundance may provide the basis for a salmon habitat management strategy in areas undergoing rapid urbanization. Identification and the application of appropriate protective or restorative measures to those areas responsible for supporting abundant salmon populations could play a major role in the maintenance and recovery of salmon populations.

Coho salmon have been shown to move considerable distances during freshwater rearing, sometimes utilizing habitats far downstream from the location where the adult fish spawn (Groot and Margolis 1991). This fact would seem to suggest that focusing protection or restoration efforts on relatively small watersheds that are known to consistently support large numbers of spawning fish would not be an effective strategy. However, we found numerous examples where sites that supported high numbers of spawning fish were found in close proximity to those with few spawners. Juvenile fish from these neighboring index sites had access to the same downstream habitats, yet there were consistent differences in the number of fish returning to spawn. These observations indicate that habitat conditions proximate to the spawning site are important in determining the ultimate success of the fish spawned at that site. The small-scale spatial variability in spawner distribution does not indicate that habitats lower in the watershed are not important for coho salmon. It has been amply demonstrated that floodplain habitats along large rivers are heavily utilized by rearing coho parr, especially during winter months (Peterson 1982), and these sites certainly deserve consideration in habitat protection and restoration plans. However, our results suggest that the quality of the habitat near the spawning area also is an important determinant of salmon abundance and these locations also should be considered in management plans.

Although comprehensive information on the distribution of salmon abundance is available for relatively few locations, procedures using remotely sensed data have recently been developed that enable the identification of sites likely to support high levels of salmon abundance. Characterization of the spatial distribution of steelhead (*Oncorhynchus mykiss*) in several major tributaries of the Willamette River in Oregon was conducted using multiple, remotely sensed databases (Steel et al. 2004). This analysis was then used to identify locations where barrier removal would generate the largest increase in steelhead abundance. A simple, yet effective process for the identification of sites that support large populations of steelhead and coho salmon utilizing digital elevation models has been developed for watersheds on the Oregon coast (Burnett et al. 2007). Application of analytical techniques like these to other areas of the Pacific Northwest could provide a good indication of where sites supporting high salmon abundance are likely to occur.

There has been considerable debate about how available resources for salmon habitat restoration and protection should be allocated in the Pacific Northwest (Independent Science Advisory Board 2003). Our analysis suggests that expending these resources in attempts to restore salmon in highly developed environments is not likely to be successful. Despite changes in rules governing development during the period from 1984 to 2001, changes in the distribution of spawning coho salmon still suggested that salmon populations declined as urban land use increased. Until more is known about the feasibility of mitigating the impacts associated with urban development, focusing freshwater habitat restoration efforts in locations where land uses are likely to remain compatible with salmon would seem to be the most efficient use of resources.

The impact of changing patterns of land use on salmon populations is not restricted to the Puget Sound region. The human population in the Pacific Northwest from northern California through southern British Columbia continues to increase. The population in Washington, Oregon, and Idaho increased from about 7.7 million in 1980 to over 10.6 mil-

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lion in 2000 and is projected to exceed 13.4 million by 2020 (US Census Bureau 2005). Successfully retaining populations of naturally spawning salmon in the region will depend on directing the limited resources available for habitat restoration and protection towards those locations that are of greatest significance to the fish.

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Influence of growth history on the accumulation of energy reserves and winter mortality in young fish

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Abstract: In seasonal environments, accumulated energy reserves are important to avoid starvation mortality during periods of low resource levels. Here we investigated patterns of energy accumulation and the importance of growth history for winter survival in young-of-the-year Eurasian perch (*Perca fluviatilis*). Under simulated winter conditions in aquaria, we showed that high winter mortality most likely relates to the depletion of energy reserves in small perch. Correspondingly, in a field study, using four lakes covering 3–6 lake-years each, overwinter survival within cohorts was positively related to individual size. However, average size in autumn did not explain the variation in overwinter survival between cohorts. Instead, we showed that seasonal growth history is an important factor. High growth rates late in season may increase cohort survival over winter, irrespective of average size, related to a positive growth-dependent increase in allocation to energy reserves when approaching winter. Mechanisms regulating within-season temporal dynamics of growth rates are therefore suggested to be important for overall cohort performance.

Résumé : Dans les environnements saisonniers, l'accumulation de réserves énergétiques est importante pour éviter la mortalité due à la famine durant les périodes de pénurie de ressources. Nous examinons ici les patrons d'accumulation d'énergie et l'importance du déroulement de la croissance pour la survie en hiver des jeunes de l'année de la perche eurasienne (*Perca fluviatilis*). Dans des conditions hivernales simulées en aquarium, nous démontrons que la forte mortalité en hiver est très vraisemblablement reliée à l'épuisement des réserves énergétiques chez les perches de petite taille. Dans une étude correspondante sur le terrain, dans quatre lacs étudiés chacun pendant 3–6 ans, la survie à l'hiver dans les cohortes est en corrélation positive avec la taille individuelle. Cependant, la taille moyenne à l'automne n'explique pas la variation de la survie à l'hiver entre les cohortes. En revanche, nous démontrons que le déroulement saisonnier de la croissance est un facteur important. Des taux de croissance élevés à la fin de la saison peuvent augmenter la survie de la cohorte en hiver, indépendamment de la taille moyenne, à cause d'une augmentation positive de l'allocation d'énergie aux réserves dépendante de la croissance à l'approche de l'hiver. Nous croyons donc que les mécanismes qui régissent la dynamique temporelle des taux de croissance au cours de la saison sont d'importance pour la performance globale de la cohorte.

[Traduit par la Rédaction]

Introduction

Seasonal climates with periods of low food production and risk of starvation induce severe constraints on individuals in terms of food intake and energy accumulation. In temperate climates, the winter season is a period with low food production, and it has been suggested that individuals need to reach a minimum size threshold before winter to survive until spring because of a positive size-dependent capacity to withstand starvation (Schultz et al. 1998; Post and Parkinson 2001; Biro et al. 2004). Furthermore, growth and energy accumulation are normally restricted to a short growth period during summer in temperate areas, and strong resource limitation may severely restrict recruitment of young-of-the-year (YOY) fish to later developmental stages because the minimum size threshold to avoid starvation mortality in winter is not reached. Consequently, low growth rates during summer may lead to total recruitment failure and ultimately population extinction due to high winter mortality among recruits (van de Wolfshaar 2006).

Smaller individuals usually experience higher overwinter mortality than do larger ones. This has been suggested to be due to size-dependent differences in ontogenetic scaling, as metabolic demands increase less than linearly with body weight, whereas accumulated energy reserves (lipids) increase linearly with body weight. Small individuals therefore spend their energy reserves at a faster rate than larger individuals and consequently are more susceptible to starvation (Post and Evans 1989; Schultz and Conover 1999; Garvey et al. 2004).

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To survive periods with strong resource limitation such as winter, allocation to energy reserves is essential (Werner and Gilliam 1984; Post and Parkinson 2001). As a result, many animals living in temperate environments have adopted strategies to avoid winter starvation by an increased energy allocation to storage (fat reserves) when approaching winter (Rogers and Smith 1993; Schultz and Conover 1999; Tracy 1999). For example, it is commonly observed in temperate lakes that YOY fish increase their lipid levels from summer to autumn (Schultz and Conover 1997; Hurst and Conover 2003; Biro et al. 2005). Energy allocation patterns are not only the result of seasonality but also depend on body size, ontogenetic stage, and resource abundance (Post and Parkinson 2001; Biro et al. 2005). Large fish have been shown to allocate relatively more energy to lipid tissues than to somatic tissues compared with small fish (Sogard and Olla 2000; Post and Parkinson 2001; Biro et al. 2005). Furthermore, the rate at which energy reserves can be accumulated may potentially depend on both individual size and growth rate, suggesting that absolute size may not be a sufficient variable to explain variation in susceptibility to winter starvation (Kirjasniemi and Valtonen 1997; Post and Parkinson 2001).

Most studies on size-dependent overwinter mortality in fish are based on the survival pattern of single cohorts in one system or only a few replicate cohorts (but see Biro et al. 2005), which is why the influence of body size on winter survival over a wider range of environmental conditions remains an open question. Here we investigated the importance of growth history for the accumulation of energy reserves and its consequences for winter survival in YOY Eurasian perch (*Perca fluviatilis*). Our study consisted of three interrelated components. We first investigated the relationship between autumn size, changes in cohort size distribution over winter, and winter survival in relation to variation in growth rates during the later part of the growth season in four lakes covering 3–6 lake-years each. Second, we studied the size dependency in growth and accumulated lipid levels in fish during summer and autumn. Finally, we experimentally studied the relationship between body length, lipid content, and starvation mortality under simulated winter conditions.

Material and methods

Eurasian perch is distributed over most of Eurasia and is often a numerically dominant species (Johansson and Persson 1986). The larvae hatch in spring in the littoral zone but soon after hatching move out to the pelagic zone to feed on zooplankton. After a gradual development into juveniles, they shift habitat back to the littoral zone where they feed on zooplankton and macroinvertebrates (Persson 1988; Byström et al. 1998, 2003). Winter mortality has been shown to be a major cause of mortality among small perch, in both Eurasian perch (hereafter only referred to as perch) (Byström et al. 1998) and in its North American sibling species, yellow perch (*Perca flavescens*) (Post and Evans 1989). Winter mortality has been suggested to result from low resource levels in winter in combination with limited ability of perch to feed during winter conditions (Lessmark 1983; Byström et al. 1998).

Lake study

The field study was carried out in four small adjacent lakes of low productivity (Lake Abborrtjärn 1, 2, 3, and 4, hereafter referred to as LAT 1, LAT 2, LAT 3, and LAT 4, respectively) in central Sweden (64°29′N, 19°26′E) during 1994–2006. Detailed information on the lakes is given in Persson et al. (1996). The lakes are ice-covered during the period October to May. All lakes have natural populations of perch. Two of the lakes are also inhabited by pike (*Esox lucius*). Densities of pike during the study period were, however, at orders of magnitude lower than that of perch (Persson et al. 1996; L. Persson, unpublished data), and visual inspection of mortality patterns revealed no evident pattern that pike lakes (LAT 1 and LAT 2) have higher YOY mortality than no-pike lakes (LAT 3 and LAT 4) (Fig. 1). As part of a long-term experiment, roach (*Rutilus rutilus*) was introduced in LAT 2 and LAT 4 in 1993–1994 and in LAT 1 and LAT 3 in 1997 (Persson et al. 2007).

Estimates of YOY perch densities were obtained from quantitative bongo trawling during the period of 5–10 July in the littoral and the pelagic habitats (for details, see Byström et al. 1998). In spring, 1-year-old perch densities were estimated based on mark–recapture methods in years when ≥60 individuals were caught during the spring electrofishing. The entire shoreline of each lake was covered, dipping the anode of the electrofishing devise every 3 m. In years when 1-year-old perch densities were too low (<60 individuals caught during electrofishing) to allow population estimates based on mark–recapture analysis, estimates were instead obtained by using regressions based on spring trap and electrofishing captures on 1-year-old perch density obtained in years when mark–recapture was possible (Table 1) (for details, see Byström et al. 2003). As we have only a few reliable estimates of autumn densities (see below), survival over winter was calculated as the estimated population numbers in May (1-year-old perch) relative to estimated population numbers in July (YOY perch). With this approach, we also included mortality in late summer. However, our data on available autumn densities suggest that the mortality after the larval period was low. In 1997, estimates on YOY perch densities in September were made in LAT 1 and LAT 4 based on the same mark–recapture methods as used to estimate densities in spring. In LAT 1, the density of YOY was estimated to be 1300 ± 400 (mean ± 1 standard error, SE) individuals per hectare in July and 2000 ± 1100 (mean ± 95% confidence interval, CI) individuals per hectare in September. In LAT 4, the density of YOY was estimated to be 17 800 ± 4 290 (mean ± 1 SE) individuals per hectare in July and 20 000 ± 9 200 (mean ± 95% CI) individuals per hectare in September. This absence of observed mortality in late summer, for the two cohorts for which we have data, suggests that mortality, if any, from July to September is low. Furthermore, our approach should only increase the possibility of making type II errors by including the variation in summer mortality considering the importance of winter mortality in our study.

In early July, most YOY perch have moved into the littoral zone (Byström et al. 2003), so samples for size and growth measurements of YOY perch during the later part of the growth season (from the beginning of August to late September) were obtained by electrofishing every 14 days

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Fig. 1. The relationship between (*a*) size in September and survival (%) of young-of-the-year (YOY) perch (*Perca fluviatilis*), (*b*) size change over winter (size in May – size in September) and survival (%) of YOY perch (July to May), (*c*) growth exponent (β) and survival (%) of YOY perch, and (*d*) the relationship between the growth exponent (β) and residuals obtained from the linear regression analysis of size change over winter and survival. $n = 18$ for all relationships. All replicates (lakes × years) are shown in all figure panels. LAT 1 (●), LAT 2 (○), LAT 3 (▼), LAT 4 (△). Solid regression lines represent significant linear relationships.

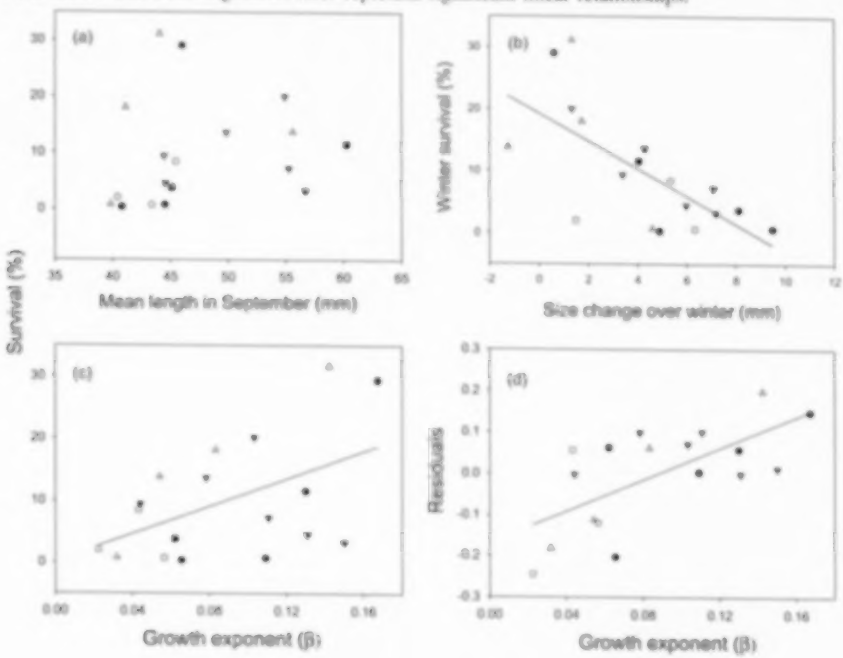

Table 1. Sampling methods, location, and time for captures of young-of-the-year (YOY) Eurasian perch (*Perca fluviatilis*) used for density, size, and lipid content calculations.

Measured variable	Sampling method	Location	Time of year
YOY density in summer	Bongo trawling	Littoral + pelagic	July
YOY density in spring (i.e., 1-year-olds)	Mark–recapture (if ≥60 individuals captured during electrofishing)	Shoreline	May
	Electrofishing + traps (if <60 individuals captured during electrofishing)	Shoreline–littoral	May
YOY size in summer–autumn	Electrofishing	Shoreline	Aug.–Sept.
YOY size in spring	Electrofishing	Shoreline	May
Lipid content summer–autumn	Electrofishing	Shoreline	Aug.–Sept.
Lipid content in winter	Traps	Littoral	Jan

along the shorelines from a boat. Also, in spring, 1-year-old perch were sampled for size measurements by electrofishing along the shoreline (Table 1). Analyses on size-selective winter survival were performed on size distributions based on electrofishing captures in September after the first growth season and in May of the following year. During this time period, the water temperature was below 10 °C, a temperature below which perch is not physiologically capable to grow in length (Lessmark 1983; Karås 1990). Size change over winter for each cohort was calculated as the difference in average length between May and September.

To investigate the influence of growth during the later part of the growth season (August–September) on accumulation of lipid mass and winter survival, a growth exponent (β) was calculated based on a power function, where t is the number of days from the first sample date in early August:

$$(1) \qquad \text{body length} = a \cdot t^{\beta}$$

Four to six measurements of mean body length (mm) per cohort and year (high β indicates high growth and low β indicates low growth in late summer) were used in the calculations. Data from YOY cohorts from all lakes and years were included when available. YOY cohorts were not included in the calculations when no fish or very few fish were caught during autumn or when sampling was too irregular (<4 sampling occasions during August–September) to obtain reliable estimates of the growth exponent (β).

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To obtain lipid levels in overwintering YOY perch in field systems, LAT 3 and LAT 4 were sampled on 18 January 1995 under the ice with minnow traps. Five minnow traps were used per lake. Traps were placed in holes (0.4 m × 0.8 m) made in the ice between 1400 and 1600 and removed between 1000 and 1200 on the following day. YOY perch were found frozen in the ice that had re-established in the holes (n = 17 and 12 in LAT 3 and LAT 4, respectively). Also free-swimming perch individuals were found in the open holes when the traps were taken up (n = 5 and 7 in LAT 3 and LAT 4, respectively). These perch were in poor condition and easily captured under the thin ice layer that had developed in the holes during night. Nine individuals were captured in the traps in LAT 4, whereas no YOY perch was captured in LAT 3. As no difference was found in lipid levels between in-frozen perch and perch captured alive (two-sample t test, P = 0.20), individuals from both groups were used for subsequent lipid analyses.

Laboratory starvation experiment

YOY Eurasian perch to be used in the laboratory experiment were collected by electrofishing in LAT 1 on 29 September 1998 at a water temperature of 6.2 °C. Captured YOY perch were brought back to the laboratory and placed into two 500 L holding tanks in a climate room. During the following week, water temperature was gradually lowered to 4 °C. Perch were fed a mixture of live zooplankton captured in a nearby pond, dominated by cladocerans, and frozen copepods. On 5 October, perch were transferred to 16 L aquaria in the climate room with a 4 h day – 20 h night light regime and a constant water temperature of 4 °C, mimicking midwinter day length and ambient deep lake water temperature conditions in this area. There was continuous freshwater flow of 32 L·day^{-1} through the aquaria, and the back and bottom of the aquaria were covered with pale green plastic plates. The fish were divided into three size classes: small, 38 ± 2 mm; medium, 45 ± 1 mm; and large, 54 ± 3 mm (mean size ± 1 standard deviation, SD; n = 50 for all size classes). For each size class, the fish were randomly placed into groups of 10 fish each, after which the groups were assigned to randomly positioned aquaria rendering five replicates with 10 individuals for each size class of perch. Introduced perch were checked for handling effects during the subsequent three days, and those not observed feeding were replaced with new individuals.

On 8 October, LAT 1 was again electrofished. No YOY perch were captured along the shoreline of the lake, suggesting that perch had migrated out to deeper parts of the lake. The feeding of perch in the aquaria was ended on the same date, and the aquaria were cleaned of food remains and faecal material before the start of the experiment on 9 October. During the experimental period, the aquaria were checked every second day for the status of the perch. Individual perch were assigned as dying when they were unable to hold an upright position or were lying on their side on the bottom of the aquaria. Dying perch were collected and their weight and length were measured prior to deep freezing. The experiment was terminated on 30 April (after 204 days), and length and weight measurements of surviving individuals were recorded prior to freezing.

Lipid analysis and energy allocation patterns

Lipid content and total dry mass were analysed using a mid-infrared transmission (MIT) spectroscopy technique (Elvingsson and Sjauna 1992). The equipment (Miris AB, Uppsala, Sweden) was calibrated for fish tissue using standard analytical methods for the compound in question (Kiessling et al. 2004; Byström et al. 2006). As individual perch did not supply enough material (>2 g) for lipid analyses, lipid analyses were performed using groups of pooled individuals. Individuals were pooled into groups so that sizes of fish in each group were as homogenous as possible. Two approaches were used to investigate energy allocation patterns. First, estimates of size-dependent lipid mass (including samples from the starvation experiment, fish sampled 18 January 1995 in LAT 3 and LAT 4, and fish sampled on 1 August and 13 September 2005 in LAT 1 and LAT 3) were obtained. Samples from the starvation experiment consisted of dying perch collected during the course of the experiment. Hence, lipid levels obtained from the experiment represent the minimum lipid level below which starvation mortality occurs. These were based on pooled samples using groups of 2–20 individuals. Each replicate represents an average of three measures of each sample through the analyser. Individual lipid content was calculated by multiplying the percentage of lipids in the sample by the mean mass of the fish used in the sample. Second, patterns of energy allocation to storage (lipid mass) versus structure (lipid-free dry mass) were compared for fast- and slow-growing YOY perch cohorts, respectively. This was done by analysing YOY perch in the size range of 48–56 mm sampled in LAT 3 during 12–13 September in two years with low growth rates late in the season (1994 and 1996, β = 0.08–0.10) and in two years with high growth rates late in the season (2005 and 2006, β = 0.15–0.20). The difference in the ratio of storage to structure was calculated as lipid mass/lipid-free dry mass (Post and Parkinson 2001), between years with low and high growth based on four samples per year, each including two to three YOY perch.

Statistical analyses

The relationships between survival, autumn size, and size change over winter for YOY perch were estimated using linear regression analyses. To examine possible interaction effects between factors, interaction terms were estimated using multiple regression analyses. All regressions were based on mean values for YOY perch within cohorts. Differences in overwinter survival of YOY perch between lakes were estimated using analysis of covariance (ANCOVA), with mean perch length as covariate. Analysis of covariance was also used to test for differences in lipid mass in YOY perch in relation to body length in LAT 1 and LAT 3 in August and September 2005. The interaction term was used to test whether the slope of the relationship between length and lipid mass differed between lakes. If the interaction term was nonsignificant, it was dropped from the model before analysing the effects of size and lake on lipid mass. Differences in lipid mass in perch at the start of the laboratory starvation experiment, in dying perch during the course of the experiment, and in perch sampled in lakes during winter were estimated using analysis of covariance, again with perch length as covariate. The temporal size dependency in

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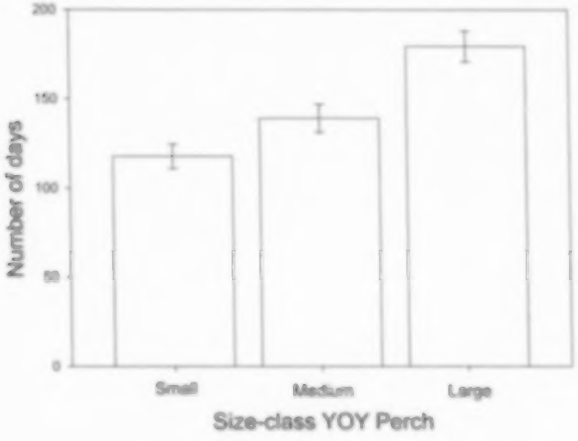
Fig. 2. Number of days until 50% mortality (means ± 1 standard error, SE) in small, medium, and large young-of-the-year (YOY) perch (*Perca fluviatilis*) in the starvation experiment. $n = 5$ for all size classes.



survival in the starvation experiment was estimated as the number of days in aquaria until 50% of the perch had been assigned the status of dying and analyzed using one-way analysis of variance (ANOVA). Proportions (survival) were arcsine square-root transformed prior to analyses. All data used were checked for normality using the Kolmogorov–Smirnov test.

Results

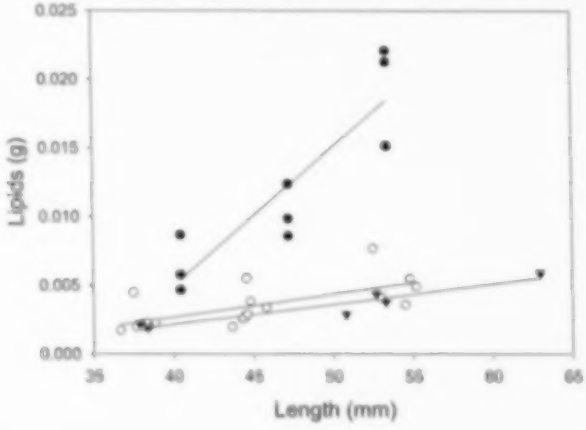
Survival, size, and growth rates

Winter survival of YOY perch did not differ between lakes (ANCOVA, $F_{[3,14]} = 0.85$, $P = 0.49$). Nor did we find a relationship between winter survival and size of YOY perch in autumn among cohorts ($r^2 = 0.09$, $P = 0.23$; Fig. 1*a*). Survival, however, was negatively related to the change in size over winter ($r^2 = 0.45$, $P = 0.002$; Fig. 1*b*), ranging from almost 0% survival in years when there was a large increase in mean size over winter up to approximately 30% survival in years when there was little change in size over winter. There was a positive relationship between growth rate late in season (β) and overwinter survival ($r^2 = 0.22$, $P = 0.049$; Fig. 1*c*). Furthermore, there was a significant interaction effect between size change over winter and growth (β) on survival ($r^2 = 0.79$, $P = 0.041$), indicated by the positive relationship between the growth exponent β and the residuals obtained from the linear regression analysis of size change over winter and survival (Fig. 1*d*). This result suggests that high growth rates during autumn decrease the severity of size-selective winter mortality, irrespective of final size in autumn. No other significant interactions were found.

Lipid levels and starvation under simulated winter conditions

Mean number of days until 50% of the perch had died in the starvation experiment increased with body size (one-way ANOVA, $F_{[2,12]} = 3.45$, $P < 0.001$; Fig. 2). At the end of the experiment, mean survival of the small, medium, and large size classes of perch in the aquaria was 2%, 12%, and 38%, respectively. Lipid levels were higher in YOY perch at the start of the experiment compared with dying perch (ANCOVA, $F_{[1,24]} = 23.6$, $P < 0.001$; Fig. 3). Lipid levels of dying perch in the experiment were similar to those of perch sampled in the lakes during winter (ANCOVA, $F_{[1,21]} = 0.005$, $P = 0.94$; Fig. 3). Lipid levels were positively related to body length both at the start of the experiment ($r^2 = 0.81$, $P = 0.001$) and for dying fish sampled during the course of the experiment ($r^2 = 0.46$, $P = 0.0014$). Lipid levels were also dependent on body size in fish sampled during winter ($r^2 = 0.89$, $P = 0.0045$). However, the slope of the relationship between size and lipid mass was steeper at the start of the experiment compared with what was the case for dying individuals sampled during the experiment (interaction term, ANCOVA, $F_{[1,24]} = 37.2$, $P < 0.001$; Fig. 3).

Fig. 3. The relationship between lipid mass and body length for young-of-the-year (YOY) perch (*Perca fluviatilis*) at the start of the starvation experiment (●) and for dying perch during the experiment (○). For comparison, the lipid mass of YOY perch caught during winter in two of the lakes (LAT 3 and LAT 4) is shown (▼). $n = 9$ for start of the experiment; $n = 17$ for starved perch; and $n = 6$ for perch sampled during winter in LAT 3 and LAT 4.



Energy allocation

Lipid mass in YOY perch was positively related to size in August, as well as in September (ANCOVA: August, $F_{[1,20]} = 79.4$, $P < 0.001$; September, $F_{[1,27]} = 38.8$, $P < 0.001$; Fig. 4). There was no difference in lipid mass between LAT 1 ($\beta = 0.10$) and LAT 3 ($\beta = 0.20$) in August, whereas lipid levels in September were higher in LAT 3 than in LAT 1 (ANCOVA: August, $F_{[1,20]} = 0.090$, $P = 0.77$; September, $F_{[1,27]} = 10.2$, $P = 0.004$). Furthermore, the slope of the relationship between length and lipid mass differed between lakes in September but not in August, being steeper in LAT 3 (interaction term, ANCOVA: August, $F_{[1,20]} = 1.87$, $P = 0.19$; September, $F_{[1,27]} = 13.6$, $P = 0.001$). More energy was allocated to energy reserves (storage in the form of lipids) compared with other tissues (structure) in years with high growth rates late in the season than in years with slow growth (two-sample t test, $P = 0.03$; Fig. 5).

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Fig. 4. The relationship between lipid mass and body length for young-of-the-year (YOY) perch (*Perca fluviatilis*) in LAT 1 (●) and LAT 3 (○) in (*a*) August and (*b*) September. Regression lines represent significant linear relationships. $n = 13$ for LAT 1 and $n = 7$ for LAT 3 in August; $n = 12$ for LAT 1 and $n = 17$ for LAT 3 in September.

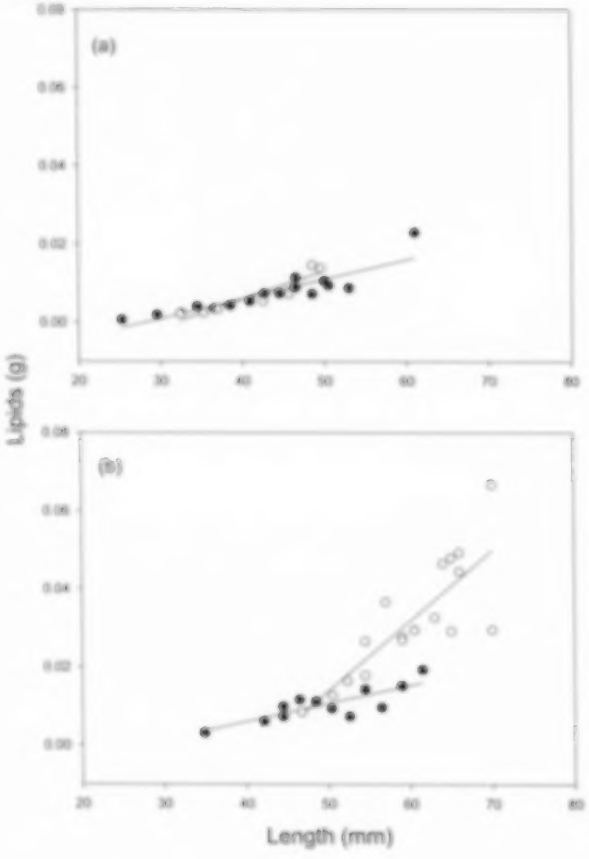
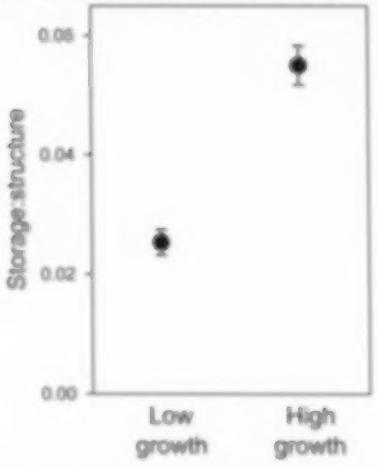


Fig. 5. The ratio of storage to structure, expressed as lipid mass to lipid-free dry mass (means ± 1 standard error, SE) in low (1994 and 1996) and high (2005 and 2006) growth years in LAT 3.



Discussion

In contrast to most studies that investigate size-dependent winter mortality in one lake over a single winter, we studied four lakes covering 3–6 lake-years each, which enabled us to investigate mechanisms behind variation in survival both among individuals within cohorts and among cohorts between years. Our results showed that overwinter survival of YOY perch was generally low but also varied substantially among lake-years. We are aware that by our approach, we included unknown mortality occurring during late summer in our estimates of winter survival. However, our two estimates of late-summer mortality (see Material and methods) in combination with earlier studies showing that cannibalism mainly affect larval, not juvenile, mortality in perch (Byström et al. 1998; Persson et al. 2000, 2004) and the lack of evidence for any density-dependent mortality in YOY perch during late summer (Byström et al. 1998) strongly suggests that starvation mortality occurring during winter is the main cause of mortality for YOY perch after the larval stages. Furthermore, in years with high YOY growth rates (which also coincide with high cannibalism-induced mortality, especially during larval stages; see Persson et al. 2004), we actually observed relatively higher survival (July to May) than in years with low YOY growth rates (coinciding with low cannibalism-induced mortality). Hence, our conclusions drawn below considering mechanisms behind the observed variation, if any, in winter performance between cohorts should be conservative regarding growth-dependent effects on winter survival.

Size-dependent winter survival within YOY fish cohorts

Within cohorts, i.e., within lake-year, our results are in agreement with those of previous studies as individual size in autumn was an important determinant for overwinter survival (e.g., Post and Evans 1989; Byström et al. 1998). The negative relationship between size change over winter and winter survival indicates that high mortality among YOY perch individuals in lakes coincided with a higher die-off of small individuals versus large individuals. The results from our laboratory experiment and lipid analyses of fish sampled in the lakes during winter show that the high mortality observed in lakes during winter most likely relates to the depletion of energy reserves, i.e., starvation mortality, as lipid levels in fish sampled during winter were similar to the minimum lipid level needed for survival derived from the experiment. In the laboratory, large individuals with more lipid mass also survived for a longer time and suffered less from starvation mortality than did small individuals, indicated by the positive relationships between size and days of survival and size and the amount of accumulated energy reserves in the form of lipids observed at the onset of winter. Hence, the likelihood of escaping starvation during winter depends both on the accumulation of lipids during the growth season and the duration of winter (cf. Sogard 1997; Post and Parkinson 2001; Biro et al. 2004). Therefore, within YOY perch cohorts, individual size is an important factor influencing survival rates over winter, in turn, suggesting size-selective starvation to be a major selective force.

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Cohort growth trajectories, overwinter survival, and recruitment variation

In contrast to the results at the within-cohort level, we found no evidence for a size dependency in winter survival among cohorts (between years and lakes), suggesting that factors other than individual size need to be taken into account to explain variation in overwinter survival. Growth rates of YOY cohorts have been shown to be an important factor in explaining survival patterns over the first growth season in fish (Miller et al. 1988; Byström et al. 1998). In our study, we show that variation in growth rates, independent of absolute autumn body size, can influence the severity of overwinter mortality among YOY cohorts. We suggest that this result relates to the positive correlation between growth rate late in season and the amount of energy accumulated at the onset of winter. For most fish species, there is little accumulation in energy reserves during summer, irrespective of growth rate (Hurst and Conover 2003; Biro et al. 2005). Instead, most energy is allocated to somatic growth in summer, and high growth rates in summer may increase survival more as a result of decreased vulnerability to predation from gape-limited predators than decreased starvation-induced mortality (Grant and Tonn 2002; Biro et al. 2005). However, less attention has been paid to the importance of temporal variation in growth rates for cohort performance and survival. The same size at the end of the growth season can be reached either by high growth rates early in summer or by high growth rates in autumn. As accumulation of energy reserves commonly increases towards the end of the season (Hurst and Conover 2003), a positive relationship between growth rate in late summer and autumn and accumulation of energy reserves could be expected. Cohorts that experience high growth rates late in the season may therefore have increased opportunities to accumulate energy reserves and consequently have an increased ability to withstand starvation mortality during winter compared with cohorts that experience high growth rates early in summer, also at equal mean size at the onset of winter. Correspondingly, we found that high growth rates late in the season was a better explanatory variable than size in autumn for explaining variation in winter starvation, which we relate to a higher accumulation to lipid reserves in years with high growth rates late in the season.

These temporal patterns of growth-dependent winter survival can have important implications for the dynamics of cannibalistic fish populations, including perch. In cannibalistic populations, a high reproductive output often coincides with a high abundance of cannibals that feed on newborns, resulting in decreased competition in late summer and autumn due to predator thinning. Few adults and hence low reproductive output may instead lead to near maximum growth rates in early YOY stages but low growth rates during later YOY stages due to high survival over summer leading to intense competition during the later part of the growth season (Persson et al. 2004; Vandenbos et al. 2006). These temporal differences in growth patterns imposed by cannibal mortality may, in turn, have great impact on cohort performance during winter as a consequence of growth-dependent accumulation of energy reserves during autumn.

In conclusion, although size in autumn is a good predictor of winter survival among individuals within a cohort, we suggest that it provides a poor explanation for differences in overwinter survival among cohorts. Instead, we show that variation in seasonal growth patterns may be an important factor explaining recruitment variation at this level. In seasonal environments, not only final size at the onset of winter, but also mechanisms regulating within-season temporal dynamics of YOY cohort growth rates, may have a large influence on later life stages, potentially affecting overall cohort performance and survival.

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Spatial and temporal variation of maturation schedules of lake whitefish (*Coregonus clupeaformis*) in the Great Lakes

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Abstract: Fish maturation schedules vary greatly among systems and over time, reflecting both plastic and adaptive responses to ecosystem structure, physical habitats, and mortality (natural and fishing). We examined maturation schedules of commercially exploited lake whitefish (*Coregonus clupeaformis*) in the Laurentian Great Lakes (Lakes Michigan, Huron, and Superior) by estimating ages and lengths at 50% maturity, age-specific maturity ogives (age-specific probability of being mature), and probabilistic maturation reaction norms (PMRNs; a metric that accounts for effects of growth and mortality). Collectively, these estimates indicated variation in maturation schedules between sexes (i.e., males tend to mature at younger ages and shorter lengths than females) and among systems (midpoint estimates of PMRNs were smallest for Lake Michigan fish, intermediate for fish in the main basin of Lake Huron, and largest for fish in Lake Huron's Georgian Bay and Lake Superior). Temporally, recent increases in age at 50% maturity in Lakes Huron and Michigan may primarily reflect plastic responses to decreased growth rates associated with ecosystem changes (e.g., declines of the native amphipod, *Diporeia* spp.). As plastic and adaptive changes in maturation schedules of fish stocks may occur simultaneously and require different management considerations, we recommend the concomitant analysis of multiple maturation indices.

Résumé : Les calendriers de maturation des poissons varient considérablement dans le temps et d'un système à un autre, ce qui reflète à la fois leurs réactions plastiques et adaptatives à la structure de l'écosystème, aux habitats physiques et à la mortalité (naturelle et due à la pêche). Nous examinons les calendriers de maturation des grands corégones (*Coregonus clupeaformis*) exploités commercialement dans les Grands Lacs laurentiens (lacs Michigan, Huron et Supérieur) en estimant les âges et les longueurs à 50 % de maturité, les ogives de maturité spécifiques à l'âge (probabilité d'être mature en fonction de l'âge) et les normes de réaction probalistes de maturation (PMRNs, une métrique qui tient compte de la croissance et de la mortalité). Dans leur ensemble, ces estimations indiquent qu'il y a une variation des calendriers de maturation entre les sexes (c'est-à-dire que les mâles tendent à arriver à maturité à un âge et une longueur inférieurs à ceux des femelles) et selon les systèmes (les estimations médianes des PMRNs sont plus basses au lac Michigan, intermédiaires chez les poissons du bassin principal du lac Huron et maximales chez les poissons de la baie Georgienne du lac Huron et ceux du lac Supérieur). À l'échelle temporelle, les accroissements récents de l'âge auquel 50 % des poissons sont matures dans les lacs Huron et Michigan peuvent représenter principalement des réactions plastiques à la diminution des taux de croissance associée aux changements dans les écosystèmes (par ex., le déclin des amphipodes indigènes *Diporeia* spp.). Puisque les changements plastiques et adaptatifs dans les calendriers de maturation peuvent survenir en même temps et nécessiter des attentions de gestion différentes, nous recommandons de faire une analyse concomitante de plusieurs indicateurs de maturation.

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Introduction

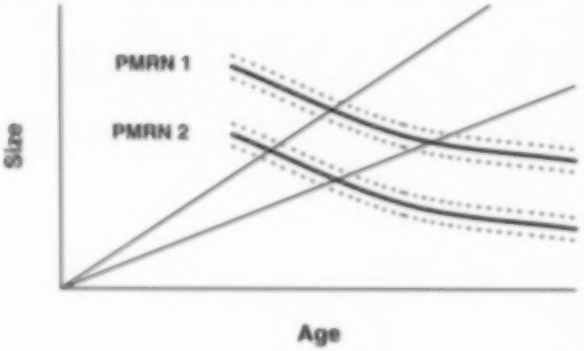
Maturation schedules constitute key demographic attributes for fisheries management. Intraspecific variation in maturation of fish might reflect both plastic (e.g., variable maturation schedules due to changes in growth or mortality rates) and adaptive (e.g., selection-induced genetic and phenotypic changes in maturation schedules) responses to various environmental factors (Law 2000). Further, widespread anthropogenic activities (e.g., fishery exploitation) have likely increased the force of genetic selection on life history traits (Stokes et al. 1993; Laikre and Ryman 1996). It is important to understand if intraspecific temporal and spatial variation in maturation schedules of fish stocks is adaptively determined, as (*i*) maturation schedules influence yield and recruitment potential (Jensen 1981), (*ii*) selection-induced variation in life history traits may be difficult to reverse (Conover and Munch 2002), and (*iii*) as suggested by Olsen et al. (2004) for Atlantic cod (*Gadus morhua*), rapid changes in genetically determined maturation schedules may be an indicator of imminent stock collapse.

Lake whitefish (*Coregonus clupeaformis*, hereafter whitefish) constitute important commercial fisheries in the Laurentian Great Lakes and numerous inland lakes (Healey 1975; Ebener 1997). Whitefish maturation schedules are known to vary dramatically among populations (e.g., Beauchamp et al. 2004). For instance, several authors have suggested that whitefish from exploited stocks grow faster, mature at younger ages and smaller sizes, and potentially have higher fecundity than whitefish from unexploited stocks (Healey 1975, 1978, 1980). Henderson et al. (1983) suggested that such changes in maturation schedules of an exploited stock might be a compensatory response (i.e., increased growth rates due to decreased population density), and Taylor et al. (1992) suggested that in addition to exploitation effects, latitudinal variation might influence size and age at maturation for Great Lakes whitefish stocks.

Many Great Lakes whitefish stocks are genetically distinct (Imhof et al. 1980; Ihssen et al. 1981; Ebener 1997), but it is unclear if such genetic variation leads to differences in maturation schedules (via genetic and environmental interactions). Intraspecific variation of maturation schedules of whitefish has been measured using traditional methods (i.e., age and length at 50% maturity; e.g., Beauchamp et al. 2004). However, such estimates can not distinguish between plastic and genetic variation (i.e., these metrics do not adequately account for the effects of growth and mortality on maturation schedules; Heino et al. 2002).

A method to estimate probabilistic maturation reaction norms (PMRNs) was developed based on the maturation reaction norm concept (Stearns and Koella 1986) to account for plastic effects of growth and mortality when depicting a population's maturation schedule (Heino et al. 2002; Barot et al. 2004*a*; Dieckmann and Heino 2007). PMRNs (usually expressed as a midpoint (at which probability of maturing = 0.5) with 95% confidence interval (CI)) represent individual variability within a population and characterize an intrinsic population-level expression of probability of maturation under different growth rates (Fig. 1; Dieckmann and Heino 2007; Kraak 2007). Conceptually, changes in growth rates (through either plastic or adaptive effects; Dieckmann and Heino 2007) may cause shifts in maturation schedules of a population with no changes to the underlying PMRN, whereas selection (via genetic or environmental factors) that alters frequency of genotypes that influence maturation schedules may alter a population's PMRN (Ernande et al. 2004; Dunlop et al. 2007). The concept of PMRN has been successfully applied to evaluate spatial and temporal variation of maturation schedules of various fish stocks (e.g., plaice (*Pleuronectes platessa*), Grift et al. 2003; smallmouth bass (*Micropterus dolomieu*), Dunlop et al. 2005).

Fig. 1. Two hypothetical probabilistic maturation reaction norms (PMRNs). The two straight lines represent fast (e.g., with a steeper slope) and slow growth trajectories. The solid and broken curves represent probability of maturing = 0.5 and 95% confidence interval (CI), respectively, at given age and size. The two PMRNs depict distinct population-level maturation schedules in response to different growth rates (i.e., the intersection of growth trajectories and PMRNs represent onset of maturation at given age and size).



It should be noted that while conceptually maturation reaction norms allow for discrimination between genetic and environmental determinants of maturation, evidence demonstrating that PMRNs are solely genetically determined is lacking. In fact, studies have reached differential conclusions regarding the relative merit of using PMRNs to explore spatial and temporal variation in genetically determined maturation schedules (e.g., Kraak 2007; Morita and Fukuwaka 2007). Herein, we apply the PMRN concept to assess potential adaptive (and not genetic) responses in maturation schedules (Olsen et al. 2004; Dunlop et al. 2005). Given that we do not know the extent to which PMRNs reflect genetic variation, similar to other authors (e.g., Ernande et al. 2004) we use the term adaptive to describe changes in traits that arise from a combination of plastic and evolutionary processes.

In this study, we aimed to evaluate spatial and temporal variation in maturation schedules of whitefish in the three upper Great Lakes (Lakes Michigan, Huron, and Superior) using three indices: (*i*) age and length at 50% maturity, (*ii*) midpoints of age-specific maturity ogives, and (*iii*) age-specific PMRN midpoints. The upper Great Lakes represent interesting systems to evaluate subtle, intraspecific variation in maturation schedules because they are large, interconnected water bodies where whitefish stocks may intermix and environmental and anthropogenic factors may influence maturation schedules of whitefish in relatively similar ways (i.e., there would likely be greater differences in environ-

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Table 1. Data sources (Michigan Department of Natural Resources (MDNR), Ontario Ministry of Natural Resources (OMNR), and Chippewa Ottawa Resource Authority's (CORA) Inter-Tribal Fisheries and Assessment Program (ITFAP)) and their temporal and spatial extent.

				Proportion of data		
Data sources	Lake	Year	*N*	May–June	July–Aug.	Sept.–Oct.
MDNR	Michigan	1989–1993	2954	0.23	0.19	0.58
(Survey data)	Superior	1971–1996	2026	0.17	0.58	0.25
OMNR	Huron (MB)	1979–2005	13294	0.43	0.04	0.53
(Survey data)	Huron (GB)	1979–2005	11033	<0.01	0.99	<0.01
CORA	Michigan	1980–2003	13915	0.23	0.26	0.51
(ITFAP data)	Huron	1980–2003	15430	0.20	0.16	0.64
	Superior	1980–2003	10909	0.27	0.28	0.45

Note: OMNR Lake Huron survey data are separated by main basin (MB) and Georgian Bay (GB).

mental and anthropogenic factors between the Great Lakes and inland lakes). Nonetheless, within these three Great Lakes whitefish experience distinct physical habitats, food web structures, and exploitation intensities (Ebener et al. 2005; Nalepa et al. 2005). As these features strongly influence size- and age-specific growth and mortality rates, it is likely that whitefish experience different selection pressures (both natural and artificial selection) among lakes.

We hypothesize that compared with fish in the other lakes, Lake Superior whitefish mature relatively late because of their historically slow growth rates (Taylor et al. 1992) caused by low water temperatures, short growing seasons, and potentially low food supply due to relatively low primary production (Barbiero and Tuchman 2001). While whitefish growth rates in Lakes Michigan and Huron have historically been higher, these rates have recently declined, likely due to both density-dependent effects (commercial catch data suggest that during recent years whitefish abundance has increased, while size at age and condition of whitefish have declined; Mohr and Ebener 2005; Schneeberger et al. 2005) and decreasing density of an important prey, *Diporeia* spp. (a high caloric-content amphipod, whose decline during the 1990s coincided with the invasion and spread of zebra mussels (*Dreissena polymorpha*); Pothoven et al. 2001; Pothoven and Nalepa 2006). Further, whitefish in Lakes Michigan and Huron have likely experienced more intense (relative to Lake Superior) but variable size-specific mortality related to sea lamprey (*Petromyzon marinus*) predation (Ebener et al. 2005) and fisheries harvest (Baldwin et al. 2002; Mohr and Ebener 2005; Schneeberger et al. 2005).

Materials and methods

Data

We analyzed two types of data sets (from three sources; Table 1) that contain information on total length, sex, and maturation status of whitefish: (*i*) fishery-independent biological survey data from the Michigan Department of Natural Resources (MDNR) and Ontario Ministry of Natural Resources (OMNR) and (*ii*) commercial catch and assessment data from Chippewa Ottawa Resource Authority's (CORA) Inter-Tribal Fisheries and Assessment Program (ITFAP).

The MDNR biological surveys were conducted annually in each lake whitefish management unit (Fig. 2) in Lake Michigan during April to October 1989–1993 using commercial trap nets (114.3 mm stretched mesh; Schneeberger et al. 2005) and in Lake Superior during May to October 1971–1996 using mostly large-mesh gill nets (114.3 mm stretched mesh) and, less frequently, graded-mesh gill nets (38.1–114.3 mm mesh in 12.7 mm increments). OMNR surveys (1979–2005) were primarily conducted in August (Georgian Bay) and June and September (southeast region (OH3 and OH4/5) of the main basin of Lake Huron; Fig. 2) using multipanel index gill nets (38.1–127.0 mm mesh in 12.7 mm increments; Cottrill and Speers 2005; Mohr and Ebener 2005). Although commercial trap net sites within each Lake Michigan management unit were randomly chosen, both agencies conducted surveys at relatively constant sites over time (e.g., OMNR surveys sampled three fixed sites in Georgian Bay and two locations in the main basin of Lake Huron; Fig. 2). Sample sizes and seasonal distributions of samples varied among the three lakes (Table 1; e.g., Lake Michigan data included a relatively high proportion of fish samples from fall (September–October)). Both agencies aged fish using scales and determined sex and maturity status of fish by internal examination of gonads. Scale aging may be biased for old (age > 7 years), slow-growing whitefish (i.e., individuals with unidentifiable annuli), but is generally accurate for young fish (age ≤ 7 years) from exploited, fast-growing populations (Mills and Beamish 1980; Mills et al. 2004).

The ITFAP data were primarily derived from fish sampled from tribal commercial catches using large-mesh gill nets (≥114 mm stretched mesh) or trap nets in the 1836-ceded waters of Lakes Michigan, Huron, and Superior (Fig. 2; Ebener et al. 2005). These data were primarily collected from May to November during 1980–2003 (similar seasonal distributions among lakes; Table 1), based on protocols that involved sampling 0.25%–0.5% of total yields from each gear and management unit (Ebener et al. 2005). Because of small sample sizes of immature fish in the commercial catches, a small percentage (23%, 29%, and 6% of samples from Lakes Michigan, Huron, and Superior, respectively) of ITFAP data was derived from whitefish assessment surveys (gear types included graded mesh survey gill nets of 50.8–152.4 mm stretched mesh in 12.7 mm increments, electrofishing, trap nets, and seines). These assessment surveys were conducted at six sites near each of three designated ports per lake (M.P. Ebener, unpublished data). While pooling data collected by different sampling gears

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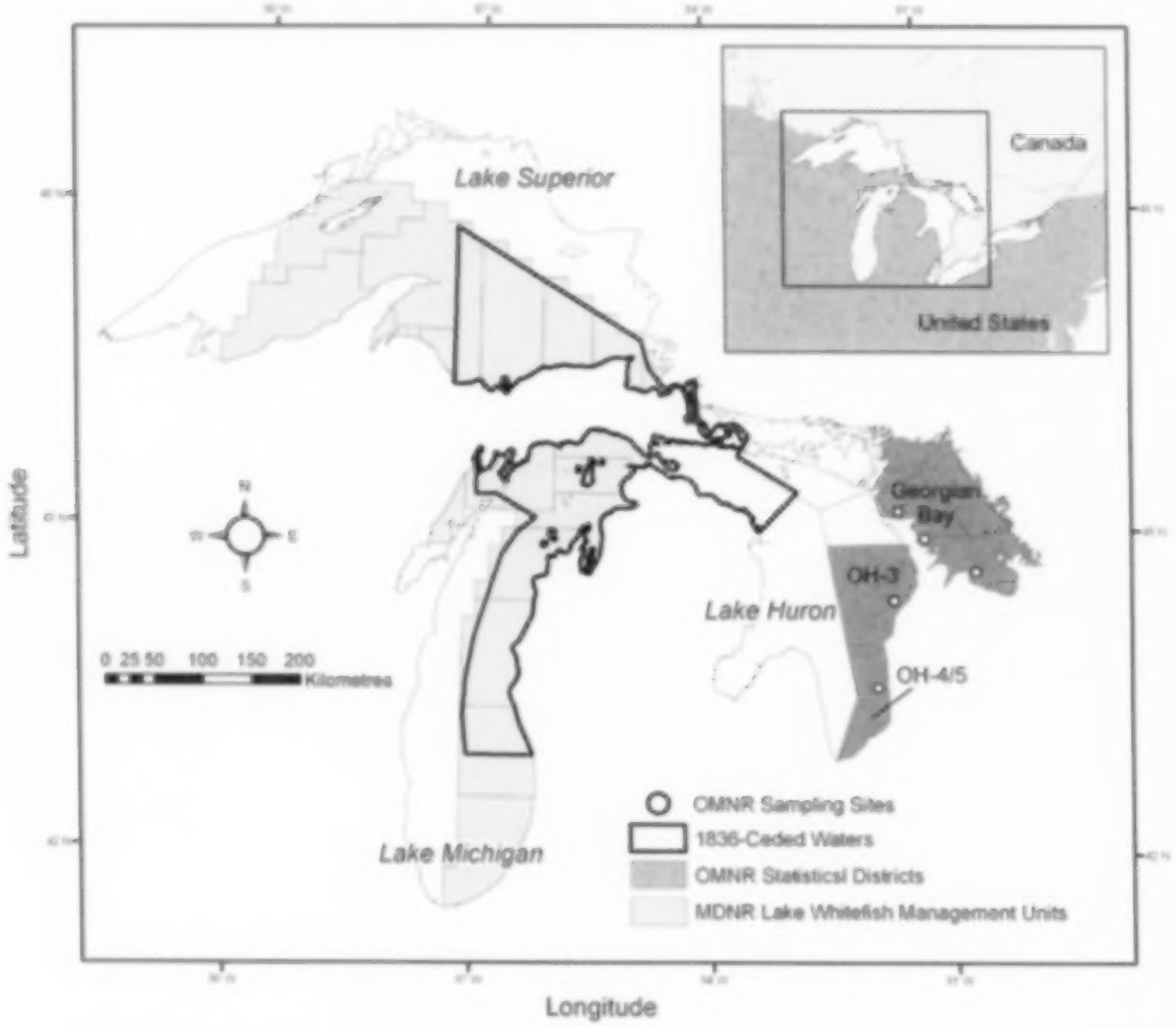
Fig. 2. Sampling areas of (*i*) Chippewa Ottawa Resource Authority's Inter-Tribal Fisheries and Assessment Program (CORA–ITFAP; data from commercial catches and assessment programs) in 1836-ceded waters (outlined with black thick lines), (*ii*) Ontario Ministry of Natural Resources (OMNR) biological surveys in Georgian Bay and southeast of main basin (including the central (OH3) and southern regions (OH4/5)) of Lake Huron (in dark gray), and (*iii*) Michigan Department of Natural Resources (MDNR) biological surveys in lake whitefish (*Coregonus clupeaformis*) management units in Lakes Superior and Michigan (in light gray). OMNR surveys were conducted at fixed sites (shown in open circles) within Lake Huron, whereas MDNR surveys were at sites randomly chosen within the MDNR lake whitefish management units.



may bias length and age distributions, the effect of gear bias was minimized when estimating PMRN midpoints (described below), because this analytic procedure facilitates analyses of biased length and age distributions (Heino et al. 2002; Barot et al. 2004*a*, 2004*b*). All of the ITFAP samples from both commercial catches and assessment programs were aged using scales. While sex of mature fish was determined via external examination during the spawning season (October and November), sex of immature fish and sex and maturity status of fish sampled during nonspawning seasons were internally examined by experienced research crews (Ebener 2005).

We used both types of data (i.e., biological survey data and ITFAP data) collected over 6 months (May to October) to compare maturation schedules of whitefish among the three Great Lakes. Moreover, the large spatial coverage of OMNR survey data allowed us to examine spatial variation in maturation schedules within Lake Huron (Georgian Bay versus main basin; Fig. 2). The inclusion of data collected over a 6-month period could lead to potential biases related to (*i*) seasonal variation in size at age and (*ii*) the differential ability to identify mature fish during various months. However, because means of size at age for age 3 and older fish were not correlated with month of capture, we believe that

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biases related to growth of fish over the 6-month period of data collection were likely minimal (an assumption borne by our results; see Discussion). In addition, mature fish were collected throughout the sampling season, and the large ITFAP data set allowed us to conduct analyses based on subsets of fish collected during August to October. We found that estimates of age-specific maturity ogive and PMRN midpoints based on data from August to October were either not significantly different or slightly smaller than estimates based on data from May to October. More importantly, spatial and temporal patterns of all maturation indices were qualitatively consistent when comparing these two temporal ranges of data (see supplemental material Table S1, Fig. S1[1]).

To compare temporal changes in maturation schedules within each lake, we aggregated cohort-specific data into two groups: fish born during or before 1990 (before 1990 cohorts) and fish born after 1990 (after 1990 cohorts). Data availability precluded us from analyzing temporal trends by annual cohort. Further, Dieckmann and Heino (2007) suggest that year-to-year variation in estimates of PMRNs can be relatively high, and thus some temporal aggregation may be appropriate. The year (1990) for division of data was chosen such that temporal subsets had approximately equal fish samples, and because the year 1990 approximates the time when zebra mussels became established and subsequently altered Great Lakes ecosystems (Mills et al. 1993; Nalepa et al. 1998). Temporal changes were determined by comparing maturation indices (see below) estimated from the two subsets. MDNR survey data were not used for temporal analyses because of insufficient temporal coverage.

Analysis

We estimated three indices of whitefish maturation schedules by sex, lake, and over time: (*i*) age and length at 50% maturity, (*ii*) midpoints of age-specific maturity ogives, and (*iii*) midpoints of PMRNs. We first estimated age (A_{50}) and length (L_{50}) at 50% maturity (i.e., the age and length at which 50% of the fish population is mature; see Beauchamp et al. 2004 for detailed formula for estimation). For each sex-, lake-, and time-specific data set, we fitted a logistic regression on data with maturity status (0 = immature; 1 = mature) as binary response and fish age or length as a predictor. The A_{50} and L_{50} were subsequently calculated by dividing the negative intercepts by the slopes of estimated logistic curves. The 95% CI of the A_{50} and L_{50} estimates were estimated using bootstrap techniques (Manly 1997). We randomly selected fish samples with replacement to generate 1000 sets of data, each of equivalent sample size as the original data set, and we fitted logistic regression on each of 1000 bootstrapped data sets to generate 1000 A_{50} and L_{50} estimates. The 95% CI of A_{50} or L_{50} was calculated as the sorted 25th (lower bound) and 975th (upper bound) values of the 1000 bootstrap estimates. Statistical differences among A_{50} and L_{50} estimates (e.g., among lakes or between time periods) were evaluated by comparing the 95% CI.

The second method for estimating maturation schedules involved fitting midpoints of age-specific maturity ogives (hereafter age-specific maturity ogives; Heino et al. 2002). This method was similar to estimating L_{50}, but the length at which probability of being mature = 0.5 was estimated separately for each age class ($L_{50,a}$; a indexes for age). Specifically, we partitioned data by lake, sex, and age and then fitted a logistic regression on each of the subsets with maturity status as response and length as a predictor:

$$(1) \qquad \text{logit}(o_a) = \ln\left(\frac{o_a}{1-o_a}\right) = \beta_0 + \beta_1 \times L_a$$

where o_a is the maturity ogive for age a, and L_a is length of fish at age a. $L_{50,a}$ was calculated by dividing the negative intercept by the slope of age-specific maturity ogive (o_a). The 95% CIs of $L_{50,a}$ were estimated using the bootstrap techniques described above.

The third method for estimating maturation schedules was the approach developed by Barot et al. (2004*a*, 2004*b*) to estimate midpoints of PMRNs. After estimating age-specific maturity ogives (from eq. 1), we then estimated the age-specific probability of maturing (m):

$$(2) \qquad m(L_a) = \frac{o_a(L_a) - o_{a-1}(L_a - \Delta L)}{1 - o_{a-1}(L_a - \Delta L)}$$

where o_a and o_{a-1} are derived from the logistic regressions fitted in the previous step. ΔL is the mean length increment from age $a-1$ to age a (i.e., $\overline{L_a - L_{a-1}}$). Probability of maturing (m) describes the fraction of fish that was immature at age $a-1$ and then grew in length (ΔL) to mature at age a. It should be noted that the validity of applying eq. 2 to estimate the probability of maturing depends on the assumption that immature and mature individuals have the same age-specific growth and mortality rates (Barot et al. 2004*a*, 2004*b*). Growth rates of whitefish decrease with increasing size (i.e., growth rates approximate von Bertalanffy growth; Mills et al. 2004). This decrease in growth may be in response to maturity, and thus our analysis could have violated the above assumption. However, Barot et al. (2004*a*, 2004*b*) demonstrated that this method might be robust even when this assumption is violated.

The probability of maturing (m) usually increased with length from zero to one and had a sigmoid shape. We followed Barot et al.'s (2004*a*) procedures to estimate the length at which $m(L_a) = 0.5$ (i.e., $Lp_{50,a}$; p indexes for PMRNs) by fitting a logistic regression with $m(L_a)$ as the response and length (L_a) as a predictor. The $Lp_{50,a}$ was calculated by dividing the negative intercept by the slope of the logistic regression (eqs. 3 and 4):

$$(3) \qquad \text{logit}\,(m(L_a)) = \beta_0 + \beta_1 \times L_a$$

$$(4) \qquad Lp_{50,a} = -\frac{\beta_0}{\beta_1}$$

The 95% CIs of $Lp_{50,a}$ were estimated using bootstrap techniques. We generated 1000 bootstrapped data sets of

[1] Supplementary data for this article are available on the journal Web site (cjfas.nrc.ca) or may be purchased from the Depository of Unpublished Data, Document Delivery, CISTI, National Research Council Canada, Building M-55, 1200 Montreal Road, Ottawa, ON K1A 0R6, Canada. DUD 3823. For more information on obtaining material refer to cisti-icist.nrc-cnrc.gc.ca/cms/unpub_e.html.

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Table 2. Age (A_{50}, years) and length (L_{50}, mm) at 50% maturity with (95% confidence interval (CI) in parentheses) of male and female lake whitefish (*Coregonus clupeaformis*) among the three upper Great Lakes based on (*i*) Ontario Ministry of Natural Resources (OMNR) and Michigan Department of Natural Resources (MDNR) surveys and (*ii*) Chippewa Ottawa Resource Authority's Inter-Tribal Fisheries and Assessment Program (ITFAP) data.

	Male			Female		
Lake	*N*	A_{50}	L_{50}	*N*	A_{50}	L_{50}
(*i*) Surveys						
Michigan	1546	2.8 (2.7, 2.9)	412 (407, 415)	1408	3.5 (3.4, 3.5)	456 (452, 461)
Huron (MB)	6952	4.2 (4.1, 4.3)	445 (442, 447)	6342	4.9 (4.8, 5.0)	484 (481, 487)
Huron (GB)	5999	4.0 (3.9, 4.1)	493 (489, 497)	5034	4.5 (4.5, 4.6)	538 (533, 543)
Superior	1044	5.3 (5.1, 5.4)	470 (463, 477)	982	5.9 (5.7, 6.1)	505 (497, 512)
(*ii*) ITFAP						
Michigan	7187	2.9 (2.7, 3.0)	410 (407, 412)	6728	3.4 (3.3, 3.5)	412 (409, 415)
Huron	9009	4.2 (4.1, 4.3)	415 (413, 417)	6421	5.1 (5.1, 5.2)	429 (427, 431)
Superior	5790	3.9 (3.8, 4.0)	414 (407, 419)	5119	4.5 (4.4, 4.6)	437 (432, 441)

Note: Estimates for Lake Huron based on OMNR survey data are separated by main basin (MB) and Georgian Bay (GB).

Table 3. Temporal variation (before vs. after 1990 cohorts) in age (A_{50}, years) and length (L_{50}, mm) at 50% maturity (with 95% CI in parentheses) of male and female lake whitefish (*Coregonus clupeaformis*) based on (*i*) Ontario Ministry of Natural Resources (OMNR) Lake Huron survey data in the main basin (MB) and Georgian Bay (GB) and (*ii*) Chippewa Ottawa Resource Authority's Inter-Tribal Fisheries and Assessment Program (ITFAP) data in the three upper Great Lakes.

		Before 1990 cohorts			After 1990 cohorts		
Gender	Lake	*N*	A_{50}	L_{50}	*N*	A_{50}	L_{50}
(*i*) OMNR							
Male	MB	4902	3.6 (3.5, 3.7)	443 (440, 445)	2050	5.5 (5.4, 5.7)	450 (445, 456)
	GB	3385	3.7 (3.6, 3.7)	483 (479, 488)	2614	4.7 (4.6, 4.8)	519 (510, 530)
Female	MB	4428	4.4 (4.3, 4.5)	489 (486, 492)	1914	5.8 (5.6, 5.9)	466 (461, 472)
	GB	2728	4.3 (4.2, 4.4)	532 (527, 538)	2306	5.1 (4.9, 5.3)	558 (544, 574)
(*ii*) ITFAP							
Male	Michigan	4388	2.7 (2.4, 2.9)	415 (411, 420)	2799	4.1 (3.9, 4.3)	406 (403, 409)
	Huron	3697	2.5 (2.2, 2.9)	412 (406, 417)	5312	5.3 (5.2, 5.3)	416 (414, 418)
	Superior	2946	3.9 (3.7, 4.1)	413 (402, 421)	2844	4.0 (3.8, 4.1)	418 (411, 424)
Female	Michigan	4003	3.1 (2.8, 3.2)	429 (425, 433)	2725	4.3 (4.1, 4.4)	403 (399, 406)
	Huron	2455	4.2 (4.0, 4.4)	444 (440, 449)	3966	5.7 (5.7, 5.8)	425 (422, 428)
	Superior	2639	4.6 (4.4, 4.7)	426 (415, 434)	2480	4.6 (4.5, 4.7)	446 (441, 451)

age a and age $a - 1$ (each of equal sample size as the original data set) and then generated 1000 estimates of $Lp_{50,a}$ (using eqs. 1–4). The 95% CI of $Lp_{50,a}$ was given as the sorted 25th (lower bound) and 975th (upper bound) value of $Lp_{50,a}$ estimates.

All three methods for estimating maturation schedules involve fitting a logistic regression on data of binary responses. As no exact statistical test exists for evaluating goodness of fit for this type of statistical model, we judged model fit by using a deviance-based test (at $\alpha = 0.05$) as well as visual inspection. In the Results, we only report estimates of all three maturation metrics generated from valid statistical models based on these criteria.

Results

Age and length at 50% maturity

A_{50} and L_{50} varied between sexes and among the three Great Lakes. In general, female whitefish had significantly greater A_{50} and L_{50} than males in all three lakes based both on survey and ITFAP data (Table 2). Among lakes, A_{50} and L_{50} of both sexes were generally lower for fish in Lake Michigan than in Lakes Superior and Huron (Table 2). Differences between Lakes Huron and Superior were less consistent (e.g., based on survey data, A_{50} estimates for both sexes were greater in Lake Superior than in Lake Huron, but ITFAP data suggested the opposite pattern). Finally, based on OMNR survey data, A_{50} tended to be lower but L_{50} was significantly greater for fish in Georgian Bay than in the main basin of Lake Huron (Table 2).

Within-lake temporal changes were evident for both A_{50} and L_{50} values (Table 3). Most noteworthy, based on both survey and ITFAP data, A_{50} values in Lakes Huron and Michigan increased significantly from before 1990 to after 1990 cohorts. On the other hand, there

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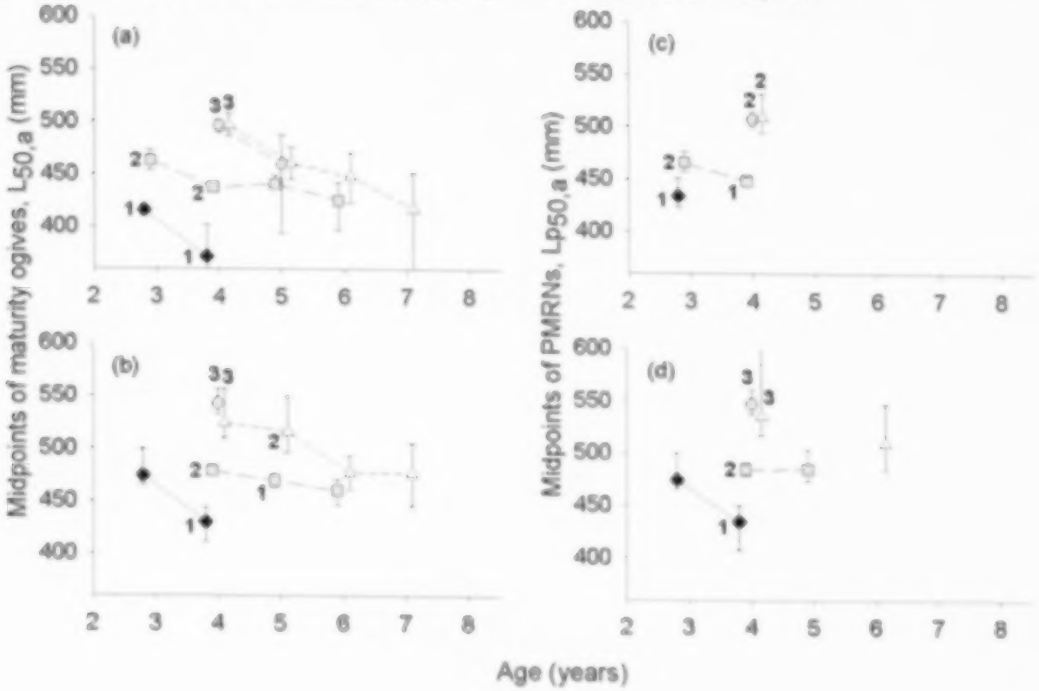
Fig. 3. Midpoints of age-specific maturity ogives ($L_{50,a}$; male (*a*) and female (*b*)) and probabilistic maturation reaction norms (PMRNs, $Lp_{50,a}$; male (*c*) and female (*d*)) for lake whitefish (*Coregonus clupeaformis*) in Lakes Michigan, Huron (main basin and Georgian Bay), and Superior based on Michigan Department of Natural Resources (in 1971–1996) and Ontario Ministry of Natural Resources (in 1979–2005) survey data sets. Error bars represent 95% confidence intervals (CIs). The labels of numbers represent significant interlake variation of age-specific estimates based on 95% CIs (i.e., if the 95% CIs of two lakes do not overlap, they are labeled with different numbers). To facilitate visual inspection, estimates for Lakes Michigan, Huron main basin, and Superior are slightly offset along the *x* axis. Black diamonds, Lake Michigan; gray squares, Lake Huron main basin; gray circles, Lake Huron Georgian Bay; open triangles, Lake Superior. The norms of $L_{50,a}$ and $Lp_{50,a}$ for a given lake are shown by connecting age-specific estimates with lines in different patterns.



were no significant changes in A_{50} values for Lake Superior, and temporal trends of L_{50} values were inconsistent (Table 3).

Age-specific maturity ogives

Estimates of age-specific maturity ogives ($L_{50,a}$) differed significantly between sexes (females > males) and among lakes at younger ages (e.g., age ≤ 6). However, at older ages, the interlake differences tended to be insignificant based on comparison of 95% CIs (CIs of estimates for older ages are relatively large because of fewer immature fish at older ages; Figs. 3*a*, 3*b*, 4*a*, 4*b*). Interlake comparisons were possible when age-specific $L_{50,a}$ and 95% CI were estimable for more than one lake. Based on survey data, $L_{50,a}$ estimates for Lake Michigan fish (for both sexes) were significantly lower than estimates for Lakes Huron and Superior (Fig. 3*a*, 3*b*). Further, $L_{50,a}$ estimates for the main basin of Lake Huron tended to be lower than both Georgian Bay and Lake Superior. There were, however, no significant differences between Georgian Bay and Lake Superior.

Based on ITFAP data, $L_{50,a}$ estimates for ages 5–6 males and ages 4–6 females were significantly lower in Lake Michigan than in the other lakes (Figs. 4*a*, 4*b*). While differences between Lakes Huron and Superior were less pronounced, age 4 female $L_{50,a}$ was significantly greater for Lake Superior than for Lake Huron (Fig. 4*b*).

Temporal changes in $L_{50,a}$ estimates were minimal. However, based on OMNR survey data, $L_{50,a}$ estimates for ages 4–5 males in Lake Huron's main basin and age 4 males in Georgian Bay increased significantly from before 1990 to after 1990 cohorts (see supplemental material Fig. S2[1]). Also, based on ITFAP data, $L_{50,a}$ estimates for age-5 females in Lake Michigan decreased significantly from before 1990 to after 1990 cohorts while no significant temporal changes were observed in the other lakes (see supplemental material Fig. S3[1]).

PMRN

Estimated age-specific PMRN midpoints ($Lp_{50,a}$) were significantly greater for females than males. Spatially, significant variation in $Lp_{50,a}$ among lakes was observed for both sexes (for ages 3–4 males and age 4 females), based on both survey (Figs. 3*c*, 3*d*) and ITFAP data (Figs. 4*c*, 4*d*). For both sexes, survey-based $Lp_{50,a}$ estimates showed that compared with fish in Lakes Huron and Superior, Lake Michigan fish matured at smaller lengths for a given age (Figs. 3*c*, 3*d*). Further, $Lp_{50,a}$ estimates for both males and females in the main basin of Lake Huron were smaller than

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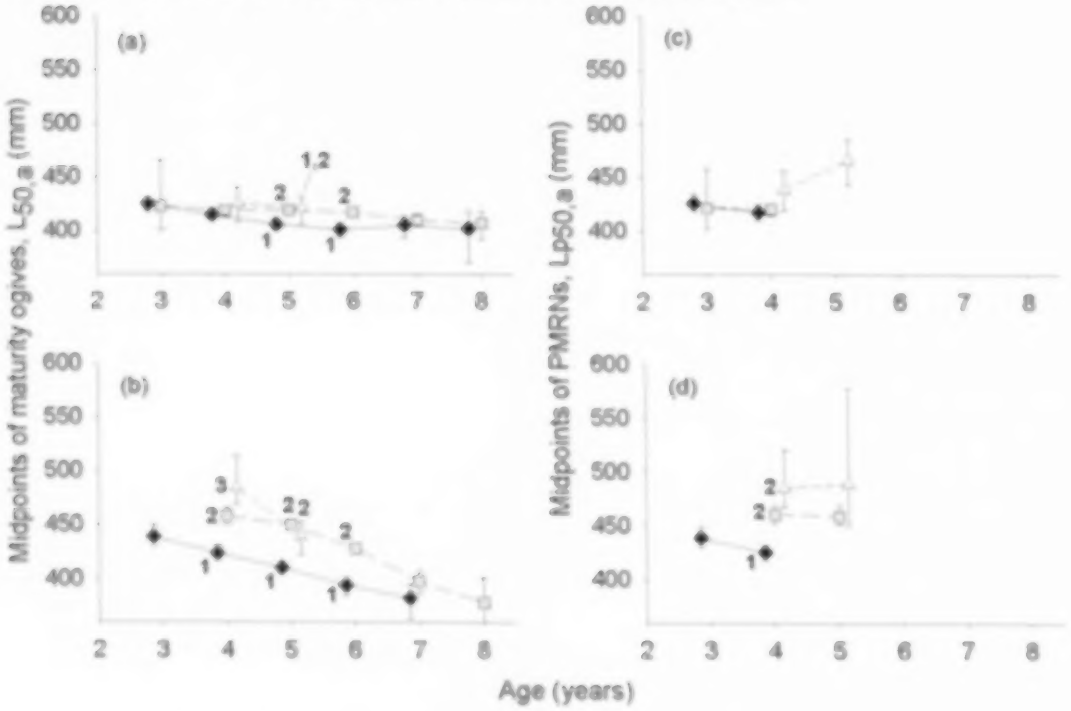
Fig. 4. Midpoints of age-specific maturity ogives ($L_{50,a}$; male (*a*) and female (*b*)) and probabilistic maturation reaction norms (PMRNs, $Lp_{50,a}$; male (*c*) and female (*d*)) for lake whitefish (*Coregonus clupeaformis*) in Lakes Michigan, Huron, and Superior based on Inter-Tribal Fisheries and Assessment Program (in 1980–2003) data sets. Error bars represent 95% CIs. The labels of numbers represent significant interlake variation of age-specific estimates based on 95% CIs (e.g., CI of a lake labeled with "1,2" overlaps with CI of lake 1 and CI of lake 2, while CIs of lakes 1 and 2 do not overlap). To facilitate visual inspection, estimates for Lakes Michigan and Superior are slightly offset along the *x* axis. Black diamonds, Lake Michigan; gray squares, Lake Huron; open triangles, Lake Superior. The norms of $L_{50,a}$ and $Lp_{50,a}$ for a given lake are shown by connecting age-specific estimates with lines in different patterns.



estimates for Georgian Bay, and there were no significant differences in $Lp_{50,a}$ estimates between fish in Georgian Bay and Lake Superior (Figs. 3*c*, 3*d*). While patterns were qualitatively similar based on ITFAP data, only $Lp_{50,a}$ for age 4 females differed significantly among lakes (Figs. 4*c*, 4*d*).

Based on OMNR survey data, $Lp_{50,a}$ estimates for age 4 males in both the main basin of Lake Huron and Georgian Bay increased from before to after 1990 cohorts (Figs. 5*a*, 5*c*). However, estimates for females in the main basin did not change significantly (Fig. 5*b*). Further, based on ITFAP data, there were no significant temporal changes in estimates of $Lp_{50,a}$ for fish of either sex in any of the lakes (Fig. 6).

Discussion

We demonstrate that maturation schedules of upper Great Lakes whitefish vary sexually, spatially, and temporally. Simultaneous analyses of two types of data (survey and ITFAP data) using three methods (age and length at 50% maturity (A_{50} and L_{50}), midpoints of age-specific maturity ogives ($L_{50,a}$), and PMRNs ($Lp_{50,a}$)) suggest that (*i*) intrinsic variation in maturation schedules exists between sexes (i.e., females tend to mature at older ages and larger sizes than males); (*ii*) whitefish of both sexes in the three Great Lakes and within Lake Huron have distinct maturation schedules, with Lake Michigan fish maturing at smaller lengths for given ages than fish in the other two Great Lakes; and (*iii*) A_{50} increased significantly from before 1990 to after 1990 cohorts in Lakes Michigan and Huron (but not Lake Superior), but these temporal shifts appear to be primarily plastic responses to dramatic ecosystem changes.

Sexual variation

Our findings of delayed maturation schedules of female whitefish are consistent with predictions based on life history theory. In general, energetic costs for reproduction are higher for female fish, and both female fecundity and egg size (and thus likely egg viability) increase with size of female whitefish (Ihssen et al. 1981). Given that there is likely a trade-off between reproductive and somatic growth, delayed maturation may increase overall lifetime reproductive success. Thus, female whitefish maturation schedules may have evolved to maximize lifetime reproductive capacity by delaying maturation (as seen in several salmonid species; e.g., Fleming and Gross 1994). On the other hand, male whitefish have seemingly low reproductive costs (i.e., minor trade-off between reproduction and somatic growth), and therefore, one might expect that male whitefish should mature at relatively small sizes and early ages.

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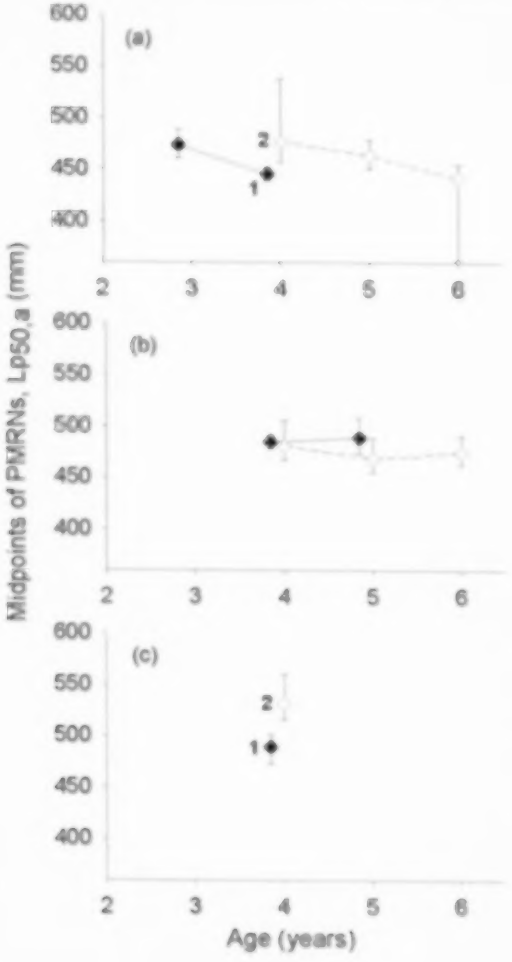
Fig. 5. Temporal (before and after 1990 cohorts) patterns (based on Ontario Ministry of Natural Resources survey data, 1979–2005) of midpoints of probabilistic maturation reaction norms (PMRNs, $Lp_{50,a}$) of male (*a*) and female (*b*) lake whitefish (*Coregonus clupeaformis*) in Lake Huron's main basin and males (*c*) in Georgian Bay. Error bars represent 95% confidence intervals (CIs). The labels of numbers represent significant temporal variation of age-specific estimates based on 95% CIs. To facilitate visual inspection, estimates for before 1990 cohorts are slightly offset along *x* axis. Solid diamonds, before 1990 cohorts; open diamonds, after 1990 cohorts. The norms of $Lp_{50,a}$ for before and after 1990 cohorts are shown by connecting age-specific estimates with lines in different patterns.



Spatial variation

Across-lake variation in $L_{50,a}$ values suggests that through adaptive processes, whitefish in the Great Lakes may express intrinsically different maturation schedules in response to long-term variable conditions among lakes (e.g., relatively high adult mortality and (or) juvenile growth rates may select for early maturation schedules; Stearns 1992). Delayed maturation schedules of whitefish in Lake Superior may in part be attributable to slow growth and relatively low mortality rates (Z = 0.5–0.7; Ebener et al. 2005). Although information on life histories of whitefish in Georgian Bay is relatively scarce, whitefish in the southern main basin of Lake Huron and throughout Lake Michigan may have experienced relatively similar selection pressures on maturation schedules, with fish in both systems likely experiencing relatively high adult mortality (Lake Michigan, Z = 0.8–0.9; Lake Huron, Z = 0.8; Ebener et al. 2005) owing to a combination of intensive harvesting, sea lamprey-related mortality, and other sources of natural mortality.

Across-system difference in maturation schedules also likely reflect plastic responses to variation in mortality rates and differences in habitats and community structure among the three Great Lakes. For example, the harvest of a large proportion of a population may affect maturation schedules of whitefish through a compensatory response (i.e., maturation at younger ages and larger size due to increased growth rates at lower population density; Jensen 1981).

Our analysis suggested that whitefish maturation schedules differ between Lake Huron's main basin and Georgian Bay (i.e., two large, distinct basins in the same lake). It is quite possible that local adaptations also lead to within-lake differences in whitefish maturation schedules at much finer spatial scales. Such differences likely exist because of (*i*) the large geographic extent and heterogeneous habitats of the Great Lakes, (*ii*) spatially variable selection pressures within these lakes (Taylor et al. 1992), and (*iii*) potential reproductive isolation among substocks.

Temporal variation

Large increases in A_{50} of whitefish from before 1990 to after 1990 cohorts in Lakes Michigan and Huron coupled with only minor shifts in estimated age-specific maturity ogive and PMRN midpoints may reflect primarily plastic, temporal changes in whitefish maturation schedules in response to dramatic, within-lake ecosystem changes. Several authors have suggested that recruitment and harvest production of whitefish in both lakes increased from the 1960s to mid-1990s as a result of sea lamprey control (Eshenroder and Burnham-Curtis 1999), decreased predation on early life stages (by alewife, *Alosa pseudoharengus*; and rainbow smelt, *Osmerus mordax*), low interspecific competition (e.g., with other coregonines; Ebener 1997), and favorable climatic conditions (Taylor et al. 1987; Freeberg et al. 1990; Brown et al. 1993). More recently (during the 1980s to 1990s), growth rates (as indicated by mean length- and weight-at-age) and body condition of whitefish have declined in Lakes Michigan and Huron, likely reflecting both intraspecific, density-dependent effects and diet shifts to energetically unfavorable prey (due to severe declines in high caloric-content *Diporeia* spp.; Pothoven et al. 2001; Mohr and Ebener 2005; Pothoven and Nalepa 2006). Decreased growth rates of whitefish in the two lakes likely led to upward shifts in the age structure of mature fish (i.e., higher A_{50} estimates as reported in our study and elsewhere; Mohr and Ebener 2005). Interestingly, as opposed to Lakes Michigan and Huron, whitefish growth rates in Lake Superior have not declined (Schorfhaar and Schneeberger 1997;) perhaps because *Diporeia* spp. densities have remained stable in this system (Scharold et al. 2004; Dermott et al. 2005; Nalepa et al. 2006), and in turn we found no signifi-

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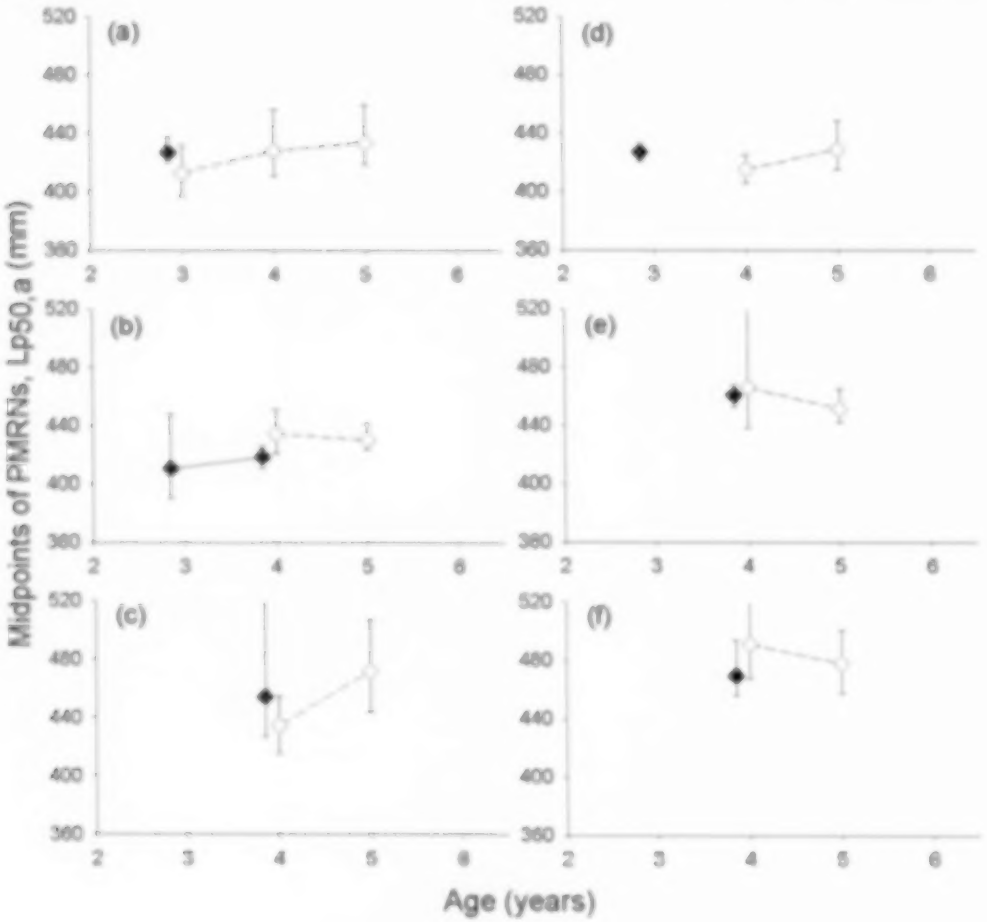
Fig. 6. Temporal (before and after 1990 cohorts) patterns (based on Inter-Tribal Fisheries and Assessment Program data, 1980–2003) of midpoints of probabilistic maturation reaction norms (PMRNs, $Lp_{50,a}$) of male (left column) and female (right column) lake whitefish (*Coregonus clupeaformis*) in Lakes Michigan (*a*, *d*), Huron (*b*, *e*), and Superior (*c*, *f*). Error bars represent 95% confidence intervals (CIs). To facilitate visual inspection, estimates for before 1990 cohorts are slightly offset along *x* axis. Solid diamonds, before 1990 cohorts; open diamonds, after 1990 cohorts. The norms of $Lp_{50,a}$ for before and after 1990 cohorts are shown by connecting age-specific estimates with lines in different patterns.



cant temporal variation in A_{50} estimates for Lake Superior whitefish.

Temporal changes in $L_{50,a}$ and $Lp_{50,a}$ within lakes were minimal. Several authors have suggested that long-term, size-selective fishery harvest may lead fish to evolve earlier age and smaller size at maturation (Stokes et al. 1993; Rochet et al. 2000; Olsen et al. 2004). While it is quite possible that Great Lakes whitefish have evolved in such a manner, we analyzed data that cover a short period relative to the history of commercial fishing (in Lake Michigan, commercial fishing began in the 1840s; Wells and McLain 1973), and thus our temporal analyses may not be able to sufficiently evaluate this hypothesis.

It should be noted, however, that survey-based estimates of $Lp_{50,a}$ values for Lake Huron male fish did change from before 1990 to after 1990 cohorts. Relative to temporal trends in fisheries pressure, Lake Huron's ecosystem structure changed dramatically from before 1990 to after 1990 (e.g., decline of *Diporeia* spp. after invasion of zebra mussels). Thus, this shift suggests that temporal variation in ecosystem characteristics may induce not only plastic, but also rapid adaptive changes in maturation schedules.

Analytical considerations

Our conclusions regarding temporal and spatial variation in both plastically and intrinsically determined maturation schedules are based on comparisons between traditional course indices of fish maturation schedules (A_{50} and L_{50}) and age-specific measures ($L_{50,a}$ and $Lp_{50,a}$; Heino et al. 2002; Barot et al. 2004*a*, 2004*b*). We suggest that these different types of indices are complementary and can collectively provide insight as to both plastic and adaptive trends in maturation schedules. The A_{50} and L_{50} metrics provide a relatively quick and simple index of maturation schedules.

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However, these estimates are sensitive to sampling biases (e.g., gear selectivity, different time of sampling, etc.) as well as growth and mortality rates of a fish stock (Heino et al. 2002). Further, on their own these indices provide limited insight as to whether variation in maturation schedules is primarily dependent on plastic or adaptive processes. Conversely, $L_{50,a}$ and $Lp_{50,a}$ are relatively unbiased by factors that alter age distributions alone. While PMRN indices are useful for comparing spatial and temporal patterns in adaptively determined maturation schedules, these indices do not allow evaluation of nongenetically determined, plastic variation of maturation schedules. We recommend that fisheries agencies should assess maturation schedules of fish stocks via these different maturation metrics to identify plastic and adaptive changes in maturation schedules that may occur simultaneously and require different management considerations.

In evaluating $Lp_{50,a}$ indices, it is important to consider the underlying assumption of such estimates (i.e., length-at-age is the primary determinant of individual maturation). Several authors have accepted this assumption and used estimates of PMRN midpoints to compare intraspecific, genetically determined maturation schedules across space and time (Grift et al. 2003; Olsen et al. 2005; Dunlop et al. 2005). A noteworthy study by Dunlop et al. (2005) compared maturation schedules of two distinct smallmouth bass populations from a common source population and revealed that while these two populations had very different maturation schedules, their $Lp_{50,a}$ values were not significantly different, thereby demonstrating the utility of this approach. Nonetheless, while length-at-age is a useful proxy of whole lifetime growth, it is clear that a variety of factors contribute to timing of maturation (Marshall and McAdam 2007; Wright 2007).

Recent studies demonstrate that using additional or different explanatory variables (e.g., weight, condition, or temperatures) to estimate PMRN midpoints may better encapsulate plastic effects on maturation (e.g., three-dimensional PMRNs; Grift et al. 2007; Kraak 2007). For instance, it is possible that weight-at-age and condition strongly influence PMRN midpoints for Great Lakes whitefish. Mature whitefish in inland lakes are known to express facultative spawning because of insufficient energy intake for maturation (Kennedy 1953). Consequently, it is possible that whitefish in poor body condition in Lakes Michigan and Huron will not spawn during successive years (Pothoven et al. 2001; Pothoven and Nalepa 2006), which may affect precision of estimates of PMRN midpoints. To explore the effects of weight-at-age on age-specific maturation of whitefish, we analyzed MDNR and OMNR survey data and estimated midpoints of PMRNs with weight (instead of length) as a predictor. These analyses suggested that PMRN metrics based on weight reveal qualitatively similar spatial and temporal patterns as estimates based on length (see supplemental material Figs. S4, S5[3]).

In addition, other partially genetically determined processes may influence the expression of maturation schedules. For example, recent studies suggest that growth rates may vary genetically within and between populations (Conover and Munch 2002; Walsh et al. 2006). As a result, variation in maturation schedules could be genetically influenced via maturation, growth, or correlated growth–maturation heritance. Nonetheless, Dieckmann and Heino (2007) suggested that population-level PMRN estimates are likely unaffected by heritable growth or correlated growth–maturation heritance provided that growth variation is primarily environmentally determined. Given that whitefish display highly plastic growth (Healey 1975, 1980; Henderson et al. 1983), it is likely that intraspecific genetic variation in growth has minimal biasing effects on PMRN estimates.

While previous research has suggested that PMRNs may be used as a sign of potential evolved changes in maturation schedules (Olsen et al. 2004), this estimate alone does not unequivocally indicate local adaptation. Further, as suggested above, evidence demonstrating that PMRNs are solely genetically determined is lacking, and studies have reached differential conclusions regarding the relative merit of using PMRNs to explore spatial and temporal variation in genetically determined maturation schedules (e.g., Kraak 2007; Morita and Fukuwaka 2007). We suggest that PMRN analysis should be considered with growth, condition, and genetic evidence to explore adaptive changes in maturation schedules (Marshall and McAdam 2007). Also, explicit investigation of the ability of PMRN to reveal genetic variation would greatly benefit future PMRN analyses.

Data considerations and conclusions

We analyzed data collected by different agencies during slightly different times of year. While the use of such variable data could introduce biases in our analyses, we believe that our conclusions regarding spatial and temporal variation of whitefish maturation schedules are at least qualitatively robust. As an example, the seasonal distributions of survey data varied among lakes (higher percentage (58%) of fish collected in fall in Lake Michigan than in Lakes Huron (30%) and Superior (25%)). These seasonal distributions would suggest that $L_{50,a}$ and $Lp_{50,a}$ estimates for Lake Michigan could be positively biased (owing to additional growth during summer and fall). However, we observed the opposite (i.e., sex- and age-specific estimates of maturity ogives and PMRNs were smallest for Lake Michigan fish), suggesting that the magnitude of differences in lake-specific maturation schedules were large enough to overcome differences in time of collection.

It is also noteworthy that we analyzed two types of data (OMNR and MDNR survey data and ITFAP data), which yielded quantitatively different results (e.g., we found that some survey-based estimates of lake-, time-, and sex-specific A_{50}, L_{50}, $L_{50,a}$, and $Lp_{50,a}$ were significantly greater than ITFAP-based estimates). Collection of these two types of data involved different agencies, time periods, sampling gear, as well as locations within the lakes. Because estimation of $L_{50,a}$ and $Lp_{50,a}$ is relatively robust to different sampling procedures (Heino et al. 2002) and invalid assumptions (e.g., adults and juveniles for given ages have the same growth increments; Barot et al. 2004*a*, 2004*b*), such differences in maturation indices between the two types of data could reflect agency biases, including the possibility that different agencies sample different whitefish substocks within a lake. However, our analyses based on different types of data led to the same qualitative conclusions regarding temporal and across-lake patterns of white-

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fish maturation schedules, suggesting that maturation schedules may differ among Great Lakes whitefish stocks and that recent within-lake shifts in maturation schedules are primarily attributable to plastic processes.

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Oxygen concentration in the water boundary layer next to rainbow trout (*Oncorhynchus mykiss*) embryos is influenced by hypoxia exposure time, metabolic rate, and water flow

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Abstract: The objective of this study was to examine the influence of hypoxia exposure time, metabolic rate, and water flow rate on the O_2 concentration in the boundary layer outside and inside the chorion of rainbow trout (*Oncorhynchus mykiss*) embryos. Oxygen consumption, growth, and dissolved O_2 in the boundary layer were measured at 15, 22, and 29 days post fertilization (dpf) from embryos reared in normoxia (O_2 concentration at 100% saturation) and chronic hypoxia (>24 h, 50% saturation) and exposed to acute hypoxia (30 min, 50% saturation). Chronic and acute hypoxia exposure decreased the dissolved O_2 in the boundary layer to the same extent at 15 and 22 dpf; however, at 29 dpf, O_2 levels were significantly lower in acute relative to chronic hypoxia. At 29 dpf, O_2 uptake per individual was significantly lower in embryos exposed to chronic relative to acute hypoxia. In addition, mass-specific O_2 uptake in chronic hypoxia-exposed embryos was ~40% less than that of controls but the same as that of acutely exposed embryos. This correlates with reduced growth in embryos exposed to chronic hypoxia. We conclude that boundary layer O_2 is lower after 30 min of hypoxia compared with 2 weeks of hypoxia simply because embryos exposed to chronic hypoxia grow slower and consume less O_2.

Résumé : Notre étude examine l'influence de la durée de l'exposition à l'hypoxie, du taux métabolique et du taux d'écoulement de l'eau sur la concentration d'oxygène dans la couche limite à l'extérieur et à l'intérieur du chorion des embryons de truites (*Oncorhynchus mykiss*). Nous avons mesuré la consommation d'oxygène, la croissance et la concentration d'oxygène dissous (DO) dans la couche limite 15, 22 et 29 jours après la fécondation chez des embryons élevés en normoxie ([O_2] à 100 % de saturation) ou en hypoxie chronique (>24 h, 50 % de saturation) ou alors exposés à une hypoxie aiguë (30 min, 50 % de saturation). Les hypoxies chroniques et aiguës causent une diminution de DO dans la couche limite de même importance 15 et 22 jours après la fécondation; cependant, 29 jours après la fécondation, les concentrations d'oxygène sont significativement plus basses lors d'une hypoxie aiguë que d'une hypoxie chronique. Vingt-neuf jours après la fécondation, l'absorption d'oxygène par individu est significativement plus faible chez les individus exposés à une hypoxie chronique plutôt qu'à une hypoxie aiguë. De plus, l'absorption d'oxygène spécifique à la masse chez les embryons exposés à une hypoxie chronique est ~40 % inférieure à celle des témoins, mais semblable à celle des embryons ayant subi une exposition aiguë. Cela s'explique par la croissance réduite des embryons exposés à une hypoxie chronique. Nous concluons que la concentration d'oxygène dans la couche limite est plus basse après 30 min d'hypoxie qu'après 2 semaines, parce que les embryons exposés à l'hypoxie chronique croissent plus lentement et consomment moins d'oxygène.

[Traduit par la Rédaction]

Introduction

Rainbow trout (*Oncorhynchus mykiss*) are relatively sensitive to hypoxia exposure (Gesser 1977). This sensitivity reaches a peak during early ontogeny, just before hatching (Rombough 1988). Understanding the ability of the developing embryos to adjust to hypoxic conditions is important for predicting the success of emerging rainbow trout populations. The physiological responses of salmonids to hypoxia are dependent on the species, age, and intensity of the hypoxia exposure (Jensen et al. 1993). Furthermore, responses to acute hypoxia may be different from those in chronic hypoxia. Several studies have focused on metabolic responses of salmonid adults to hypoxia (e.g., Dunn and Hochachka 1986; Boutilier et al. 1988; Zhou et al. 2000), but few studies are available on embryonic stages.

Previous studies have demonstrated that rainbow trout embryo survival within the redd is dramatically influenced by water O_2 levels. Youngson et al. (2004) reported that there was 83% mortality of rainbow trout embryos in redds where mean dissolved O_2 (DO) was at 28% saturation. Also, survival was significantly related to the velocity of ground-

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water in redds (Sowden and Power 1985). An assessment of a rainbow trout spawning redd found that DO concentrations ranged from ~23% to ~80% saturation (Coble 1961) and that the intergranular flow rate of water through salmonid redds was also variable from 2.7 to 4.5 mL·min^{-1}, typically averaging ~3 mL·min^{-1} (Zimmermann and Lapointe 2005). Coble (1961) has also suggested that DO concentrations and water flow rates directly impact the intragravel water of a rainbow trout redd. Therefore, in addition to hypoxia resulting from variability in DO, variability in water flow rate can also limit the delivery of O_2 to embryos.

Prehatch development represents an extremely sensitive period in trout, as organogenesis occurs between 4 days post fertilization (dpf) and hatching (Balon 1975). Any disturbances to this may result in malformation and defects that will ultimately impact the fitness of adults (Shang and Wu 2004). Specifically, the growth of embryos can be open to considerable ontogenic modification in response to habitat conditions. For instance, low DO concentrations have been shown to affect the rate of development of chum salmon (*Oncorhynchus keta*; Alderdice et al. 1958), lake trout (*Salvelinus namaycush*; Garside 1959), and Chinook salmon (*Oncorhynchus tshawytscha*; Silver et al. 1963) embryos. Furthermore, during periods of low O_2 availability, it is essential that fish embryos maintain respiratory homeostasis and sufficient gas exchange required for the increase in growth, development, and O_2 consumption (M_{O_2}) associated with embryogenesis. It is known that rainbow trout adults show a metabolic depression after 24 h in mild hypoxia (~50% O_2 saturation) (Boutilier et al. 1988). Also, nonsalmonid embryos such as zebrafish (*Danio rerio*) decrease M_{O_2} in response to severe (~30% O_2 saturation) acute (20 min) hypoxia (Barrionuevo and Burggren 1999). The ability of trout embryos to depress M_{O_2} has not been well studied. Thus, it is necessary to determine how ontogeny, with its periods of variable sensitivity, influences physiological tolerances and responses to short- and long-term mild hypoxia in the trout embryos.

The boundary layer consists of an O_2-depleted region of stagnant water owing to O_2 consumption by the embryos, creating a significant O_2 gradient (Pinder and Feder 1990). The O_2 boundary layer around developing embryos depends on both the supply of and the demand for O_2. Ciuhandu et al. (2007) showed that increased O_2 demand in later stages of trout embryogenesis decreased O_2 levels in the boundary layer, but they did not directly measure O_2 consumption. A decrease in O_2 supply (e.g., hypoxia) may further reduce the amount of O_2 in the boundary layer available to the embryos. Ciuhandu et al. (2007) found a decrease in DO and the DO gradient in the boundary layer of rainbow trout embryos in acute hypoxia (30 min, 35% saturation). However, in the Ciuhandu et al. (2007) study, DO inside the chorion (perivitelline fluid) was not measured, nor was the influence of prolonged hypoxia exposure investigated.

In the present study, we considered the impact of a mild hypoxia (50% saturation) that may be frequently encountered by salmonid embryos in their natural environment. We initiated chronic hypoxia exposure at the eyed stage (14 dpf) because it correlates with the onset of simple circulation (Rombough 1997), a juncture in embryonic development coinciding with a gradual increase in convective O_2 transport and its altering effects on diffusion properties and critical DO levels (Alderdice et al. 1958). For the purpose of this study, chronic hypoxia is defined as the condition in which the embryos are maintained in water with an O_2 concentration at 50% saturation for a period greater than 24 h.

There were four hypotheses addressed in this study. The first hypothesis was that the distribution of DO inside the chorion and the boundary layer O_2 gradient would be influenced by environmental DO. We predicted that the DO levels inside the chorion and in the boundary layer would be lower when exposed to acute and chronic hypoxia at three sampling times during the course of development. This was tested by measuring the DO profile inside the chorion and in the boundary layer at 15–16, 22–23, and 29–30 dpf in normoxia (100% O_2 saturation) and acute (30 min) and chronic hypoxia. The second hypothesis was that hypoxia would depress O_2 uptake. We predicted that acute and chronic hypoxia exposure would reduce M_{O_2} below the control rate in normoxia during late embryonic development but not earlier because of the low O_2 requirements of embryos early in development. To test this, M_{O_2} was measured at the same sampling times as above and with the same O_2 treatments. The third hypothesis was that hypoxia influences the growth of rainbow trout embryos throughout development. We predicted that there would be a decrease in growth in response to hypoxia in chronic but not in acute exposure at all sampling times, with the most impact on growth late in development because of high O_2 requirements of embryos late in development. To test this, the embryonic dry mass of embryos was determined at the same sampling times and with the same O_2 treatments as above. The fourth hypothesis was that water flow rate would affect the DO inside the chorion and in the boundary layer outside the chorion. We predicted that a decrease in water flow rate would lower the DO concentration inside the chorion and in the boundary layer outside the chorion. To test this, DO levels in the boundary layer and inside the chorion of 28 dpf embryos were measured at water flow rates of 3 and 6 mL·min^{-1}.

Materials and methods

Experimental animals

Rainbow trout embryos were obtained from Rainbow Springs Trout Farm in Thamesford, Ontario, on the day of fertilization and transported to the Hagen Aqualab, University of Guelph (Guelph, Ontario). Embryos were held in mesh-bottom Heath trays (length 62.9 cm × width 52.1 cm × depth 6 cm) throughout the incubation period shielded from light with a continuous flow (4 mL·min^{-1}) of local well water (10 °C, 10 mg O_2·L^{-1}, pH 7.9, water hardness 411 mg·L^{-1} as $CaCO_3$, ion concentrations (mmol·L^{-1}): 2.6 Ca^{2+}, 1.5 Cl^-, 1.5 Mg^{2+}, 0.06 K^+, and 1.1 Na^+). Separate batches of embryos were used for series I and series II, II, and IV. For each batch, eggs were obtained from three different females and these were mixed together with sperm from multiple males. The resultant embryos were randomly divided into the treatment and control groups. Therefore, there should be no differences between treatment and control in embryo quality. Hatching occurred between 29 and 32 dpf and there was no difference between treatment and control groups in the time to hatch.

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Experimental protocols

There were four series of experiments conducted: series I, DO in the boundary layer during development and hypoxia; series II, O_2 consumption (M_{O_2}) during development and hypoxia; series III, growth rate and hypoxia; and series IV, DO in the boundary layer with changes in water flow rate. In series I, II, and III, measurements were taken after acute (30 min) and chronic hypoxia exposure. Embryos at the eyed stage (14 dpf) were randomly divided into two groups and chronically reared at either normoxia (100% O_2 saturation) or hypoxia. Equilibrating the input of N_2 gas into a header tank (~50 L) established hypoxic water flowing in the Heath tray for the duration of the chronic hypoxia exposure. DO levels were monitored periodically and were always between 95% and 100% for the normoxic group and between 48% and 52% for the hypoxic group. For series I, II, and III, embryos were sampled at the following times: 15–16 dpf (eyed stage), 22–23 dpf, and 29–30 dpf (just before hatching). For simplicity, these times have been designated as 15, 22, and 29 dpf in subsequent referrals.

Series I: O_2 gradient with sampling time and hypoxia

Oxygen microelectrodes were used to measure DO profiles in the boundary layer and inside the chorion of rainbow trout embryos as described by Ciuhandu et al. (2007). In brief, a syringe with a 28-gauge needle was attached to a micromanipulator (Brinkmann, Germany) and used to pierce the chorion. Once penetrated, a very small amount of food colouring was introduced to mark the location of the puncture. The needle was then replaced with an O_2 electrode (model OX25; Unisense A/S, Aarhus, Denmark) connected to a picoammeter (model PA2000; Unisense A/S). DO was measured ~100–200 μm inside the chorion in the perivitelline fluid. As the penetration of the chorion could have disturbed the boundary layer inside the chorion, only one measurement was taken inside the chorion (Ciuhandu et al. 2007), and for consistency, this was completed immediately after the chorion was pierced at the same location and distance from the chorion for each embryo. For each embryo, DO was also measured at the outside surface of the chorion and at 100 μm increments away from the surface of the chorion up to 1000 μm to accurately demonstrate the size of the boundary layer.

The DO concentration in the boundary layer was measured before the chorion was pierced to prevent disturbances to the boundary layer. These measurements were taken in a Plexiglas chamber (length 7.5 cm × width 3.3 cm × depth 2 cm) supplied with flowing well water at 10 °C. The water flow rate was controlled using a variable-flow peristaltic pump (model 3385; Fisher Scientific, Pittsburgh, Pennsylvania). Calibrated ratios of air and N_2 were mixed using a Wösthoff pump (Calibrated Instruments Inc., Ardsley, New York) and bubbled into a glass reservoir with a water jacket maintained at 10 °C by a circulating water bath (Isotemp 3016; Fisher Scientific). The O_2 microelectrode was used to measure DO profiles in the boundary layer of embryos at the three sampling times in embryos exposed to acute hypoxia, chronic hypoxia, and normoxia. All measurements were on separate embryos (n = 6 for each sampling time). The O_2 gradient was calculated as the O_2 concentration in the free stream minus the O_2 concentration in the perivitelline fluid. Water flow rate during measurements was 4 mL·min^{-1}. Measurements were taken 30 min after the embryo was placed in the sample chamber to allow adequate time for the boundary layer to form.

Series II: M_{O_2} with development and hypoxia

Closed respirometry was used to measure M_{O_2} in normoxia, acute hypoxia, and chronic hypoxia at three sampling times (as above). Embryos were placed in water-jacketed (10 °C) glass respirometers (for setup details, see Ninness et al. 2006). For each respirometer, three embryos were used at 15 dpf and two embryos were used at 22 and 29 dpf (n = 8 at each sampling time). The chambers were filled with autoclaved well water: 100% O_2 saturated (normoxia) and 50% O_2 saturated (acute or chronic hypoxia). DO levels in the respirometers were monitored continuously for 1.5 h after an initial 30 min chamber acclimation period. The water O_2 content did not fall below 70% saturated in normoxia or below 30% saturated in hypoxia during this time period. Measurements were made using automatic, temperature-compensated DO sensors (Clark-type electrodes) with built-in thermistor and amplifier connected to a LabPro serial interface and recorded on a computer using LoggerPro software (version 3.4.2; Vernier Software and Technology, Beaverton, Oregon). The O_2 probes were calibrated daily. Preliminary trials were conducted without embryos in the respirometers for 2 h to determine background O_2 uptake or sensor O_2 uptake (negligible). M_{O_2} was calculated using a formula previously described by Ninness et al. (2006). M_{O_2} is presented per individual (nanomoles O_2 per minute per individual) and mass (micromoles O_2 per minute per gram dry mass).

Series III: growth rate and hypoxia

To measure and compare growth rate in embryos exposed to normoxia and acute and chronic hypoxia, embryos from series II were dechorionated and the embryonic proper was separated from the yolk sac by hand using fine forceps. The embryonic proper was oven-dried (~96 h at 50 °C) to a constant mass (milligrams). The embryonic body dry mass was pooled for each treatment and divided by the number of embryos used in each treatment to determine dry mass per individual (n = 8 at each sampling time).

Series IV: O_2 gradient with changes in water flow rate

The O_2 microelectrode was used to measure DO profiles in the boundary layer as described above. DO measurements were taken at 28 dpf in normoxic water on seven embryos at two different flow rates: 3 and 6 mL·min^{-1}. Over this range of flow rates, the linear relationship between water velocity and flow rate in the sample chamber was represented by the following equation: velocity (cm·s^{-1}) = –0.078 + 0.040 × flow (mL·min^{-1}). Velocity was measured by timing the movement of dye in the free stream near the embryo in the sample chamber. Separate embryos were used for all measurements. This experiment was only completed on embryos under normoxic conditions, as it was not possible to maintain 50% O_2 saturation in the water if the flow through the system was varied from 4 mL·min^{-1}. In addition, these experiments were not completed at a flow rate of zero, as there

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has to be water flow through the experimental setup for temperature and O_2 levels to be maintained.

Statistical analyses

A general linear model analysis of variance (GLM-ANOVA) was performed to test for differences between normoxia and acute and chronic hypoxia boundary layer DO curves (pairwise) outside the chorion at each sampling time (series I). The same procedure was performed to test for differences between the boundary layer DO curves (series IV) outside the chorion at the two flow rates (3 and 6 mL·min^{-1}). Differences between DO values inside and at the surface of the chorion were analyzed using paired Student's t tests (series IV). A one-way ANOVA was performed to analyze differences in DO under the chorion between normoxia and acute and chronic hypoxia for each sampling time (series I) and between the two water flow rates (series IV). Differences in O_2 gradients (series I) were tested with a two-way ANOVA (treatment, sampling time, and interaction). The effect of acute and chronic hypoxia on M_{O_2} (series II) was tested with a three-way nested ANOVA (treatment, sampling time, respirometer, and interaction). Differences in growth owing to acute and chronic hypoxia exposure (series III) were analyzed using a two-way ANOVA (treatment, sampling time, and interaction).

All statistical analyses were performed using Minitab version 15. In all analyses, Tukey tests were used to identify the differences when significant differences were found ($p < 0.05$). Results are presented as means ± SE of the mean.

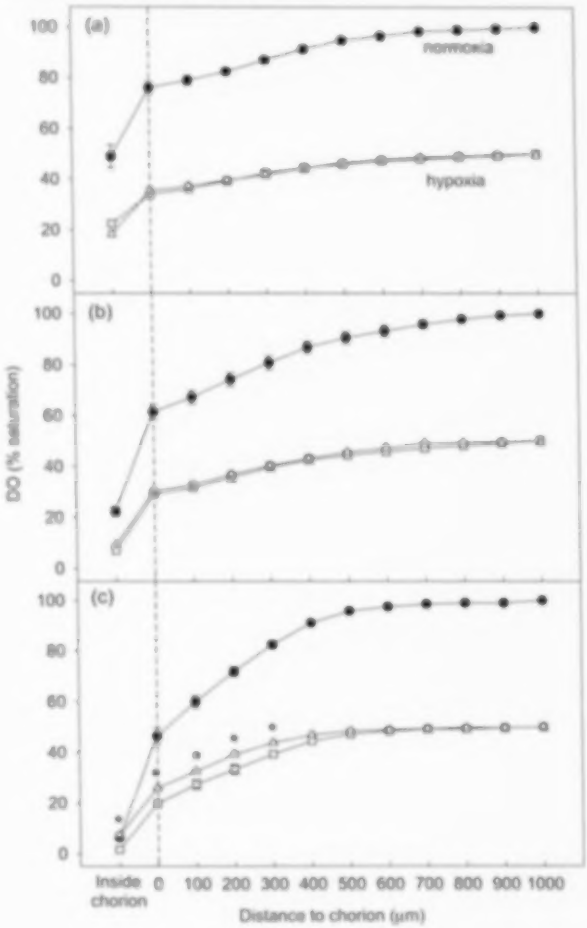
Results

Series I: O_2 gradient with sampling time and hypoxia

Oxygen concentration gradually decreased in a nonlinear fashion from the free stream to the chorion (Fig. 1). At 15, 22, and 29 dpf, the DO in the boundary layer curve was significantly decreased with acute and chronic hypoxia compared with that under normoxic conditions (Fig. 1). There was no significant difference between DO in the boundary layer curve in acute and chronic hypoxia at 15 dpf (Fig. 1*a*) and 22 dpf (Fig. 1*b*). On the contrary, at 29 dpf, the DO in the boundary layer curve in chronic hypoxia was significantly higher than that of acute hypoxia (Fig. 1*c*). Overall, the boundary layer effect on the distribution of DO (treatment and distance to chorion interaction) was the same for the two hypoxia exposures at 15 dpf (Fig. 1*a*) and 22 dpf (Fig. 1*b*) but different at 29 dpf (Fig. 1*c*). There were significant changes in the O_2 gradients with sampling time and treatment (Fig. 2). The O_2 gradient increased with sampling time in normoxia and acute hypoxia but only between 15 and 22 dpf in chronic hypoxia. The O_2 gradient was significantly less in acute and chronic hypoxia than in normoxia at each sampling time. At 29 dpf, the O_2 gradient in chronic hypoxia was significantly lower than that in acute hypoxia (Fig. 2).

At 15 and 22 dpf, DO values inside the chorion (~20% and ~8% saturation, respectively) for both acute and chronic hypoxia did not differ from one another significantly, but both DO values were significantly lower relative to DO values inside the chorion in normoxia (Figs. 1*a* and 1*b*). At 29 dpf, the DO inside the chorion in normoxia (~6% saturation) was significantly higher than DO inside the chorion in acute hypoxia (~2% saturation) (Fig. 1*c*). Also, DO inside the chorion during chronic hypoxia exposure was on average 78% higher compared with that in acute hypoxia but did not significantly differ from DO inside the chorion in normoxia (Fig. 1*c*).

Fig. 1. Boundary layer dissolved oxygen (DO) profile of rainbow trout (*Oncorhynchus mykiss*) embryos in normoxia (solid circles), acute (shaded squares), and chronic hypoxia (open triangles) (50% saturation, 10 °C) at three developmental times: (*a*) 15, (*b*) 22, and (*c*) 29 days post fertilization (dpf). Water flow rate was set to 4 mL·min^{-1}. The boundary layer curve was decreased with hypoxia at 15 and 22 dpf, but at 29 dpf, the DO curve in chronic hypoxia was significantly higher than that of acute hypoxia. An asterisk indicates a significant difference in DO between embryos chronically exposed to hypoxia and those acutely exposed to hypoxia ($p < 0.05$). The vertical broken line indicates the chorion surface. Values are means ± SE of the mean ($n = 6$).



Series II: O_2 consumption with sampling time and hypoxia

Sampling time had a statistically significant effect on M_{O_2}

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Fig. 2. Boundary layer gradient of rainbow trout (*Oncorhynchus mykiss*) embryos in normoxia (solid circles), acute (shaded squares), and chronic hypoxia (open triangles) (50% saturation, 10 °C) at three developmental times: 15, 22, and 29 days post fertilization (dpf). The gradient (% O_2 saturation) was calculated from boundary layer curves in Fig. 1 as the difference between the DO in the free stream and the DO in the perivitelline fluid. Values are means ± SE of the mean. The O_2 gradient was less in hypoxia than in normoxia at each developmental time. At 29 dpf, the O_2 gradient in chronic hypoxia was significantly lower than that in acute hypoxia.

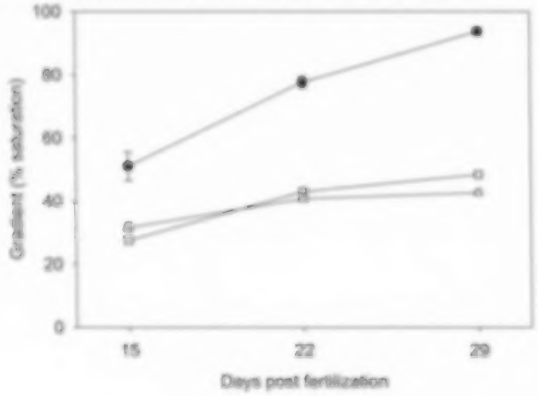
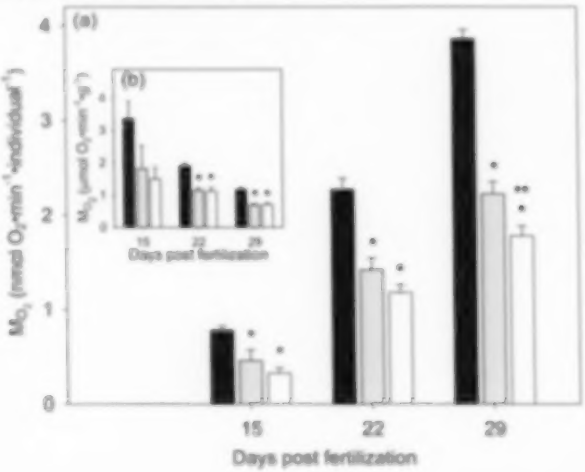


Fig. 3. Oxygen consumption of rainbow trout (*Oncorhynchus mykiss*) embryos (*a*) per individual and (*b*) mass-specific in normoxia (solid bars), acute (shaded bars), and chronic hypoxia (open bars) (50% saturation, 10 °C) at three developmental times: 15, 22, and 29 days post fertilization (dpf). A single asterisk indicates a significant difference from normoxia, and double asterisks indicate a significant difference from acute hypoxia ($p < 0.05$). Values are means ± SE of the mean ($n = 8$).



per individual (Fig. 3*a*). In normoxia, M_{O_2} per individual increased fivefold between 15 and 29 dpf. Similarly, M_{O_2} per individual in acute and chronic hypoxia significantly increased during development but overall remained lower than normoxic values.

Treatment had a significant effect on M_{O_2} per individual (Fig. 3*a*), and this effect was dependent on development. Hypoxic conditions (acute and chronic) significantly decreased M_{O_2} per individual at all sampling times. At 29 dpf, M_{O_2} per individual in chronic hypoxia was 54% lower relative to normoxia and 20% lower relative to acute hypoxia (Fig. 3*a*). There was a significant decrease in mass-specific M_{O_2} in embryos exposed to hypoxia relative to normoxia at 22 and 29 dpf but not at 15 dpf (Fig. 3*b*).

There is no significant source of variation in M_{O_2} produced among different respirometers within each O_2 treatment.

Series III: growth rate and hypoxia

Overall, growth increased throughout embryonic development for each treatment (Fig. 4). In addition, hypoxic conditions did not have an effect on growth at 15 and 22 dpf (Fig. 4). However, at 29 dpf, the embryonic body dry mass of embryos reared in chronic hypoxia was 24% lower than of those reared in normoxia.

Series IV: O_2 gradient with changes in water flow rate

Under normoxic conditions, a decrease in water flow rate from 6 to 3 mL·min^{-1} resulted in a statistically significant lower boundary layer DO curve outside the chorion (Fig. 5). There was a statistically significant difference between the DO inside the chorion and at the chorion surface for both flow rates. The DO inside the chorion at 3 mL·min^{-1} was significantly lower by 56% compared with the DO inside the chorion at 6 mL·min^{-1}.

Discussion

Before the onset of convective O_2 transport, the aerobic requirements of embryos are met solely through O_2 diffusion (Krogh 1941). Water convection past the respiratory surface is not initiated until several days after hatching (Rombough 1988), but a rudimentary circulatory system is in place at the eyed stage (~14 dpf, 10 °C; Vernier 1969). We show here that body mass and O_2 uptake increased by 12- and 5-fold, respectively, over this 2-week period between 15 and 29 dpf, in agreement with previous reports (Rombough 1986). Thus, as the demand for O_2 increases multifold, changes in the supply of O_2 (i.e., hypoxia) should result in a larger impact on O_2 levels in the microenvironment next to older compared with younger embryos (hypothesis 1). Indeed, our results show that the DO inside the chorion (perivitelline fluid) becomes progressively lower during both acute and chronic hypoxia from 15 dpf (~20% saturation) to 22 dpf (~8% saturation) and both values are lower than control values. At 29 dpf, however, we were surprised to see that the DO inside the chorion of embryos in chronic hypoxia was about the same as control values (~6% saturation), significantly higher than respective values in embryos exposed to only 30 min of hypoxia (~2% saturation). Interestingly, the M_{O_2} of embryos chronically exposed to hypoxia was ~54% lower that of the control embryos at day 29. The fact that the DO inside the chorion of both of these groups was the same at this time indicates that the lower M_{O_2} is measurably affecting DO concentrations.

We predicted that acute and chronic hypoxia exposure would depress O_2 uptake during late embryonic develop-

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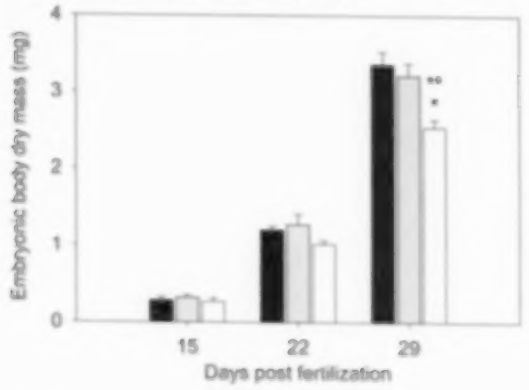
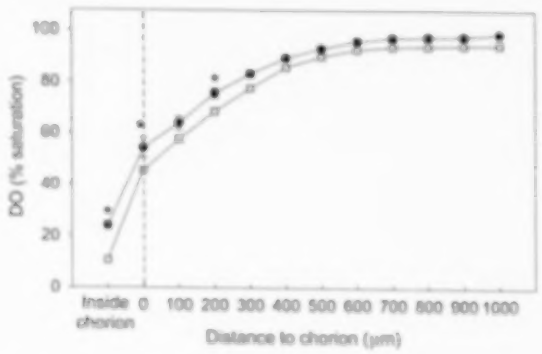
Fig. 4. Individual dry mass of rainbow trout (*Oncorhynchus mykiss*) embryos in normoxia (solid bars), acute (shaded bars), and chronic hypoxia (open bars) (50% saturation, 10 °C) at three developmental times: 15, 22, and 29 days post fertilization (dpf). A single asterisk indicates a significant difference from normoxia, and double asterisks indicate a significant difference from acute hypoxia ($p < 0.05$). Values are means ± SE of the mean ($n = 8$).



ment but not earlier because of the low O_2 requirements of the younger embryos (hypothesis 2). The data do not fully support this hypothesis because M_{O_2} per individual was depressed in all embryos, regardless of developmental stage. Furthermore, in 29 dpf embryos, M_{O_2} per individual in chronic hypoxia was significantly lower than comparable values for the acute hypoxia group. This demonstrates that O_2 demands were lower in the embryos exposed to chronic hypoxia compared with acute hypoxia, and therefore, DO inside the chorion was higher in chronic relative to acute hypoxia. The higher DO inside the chorion resulted in the observed decrease in the O_2 gradient in chronic hypoxia. Is the lower M_{O_2} in the chronic hypoxia group due to a deeper metabolic depression or to mass differences between embryos? The results show that chronic hypoxia depressed growth rate and the 29 dpf embryos attained a smaller mass relative to the other two groups, normoxia and acute hypoxia. Thus, the mass-specific M_{O_2} values for the chronic hypoxia group are not different from those for the acute hypoxia group. We can conclude, therefore, that O_2 levels in the fluid surrounding the 29 dpf embryos were higher after 2 weeks compared with 30 min of mild hypoxia because embryos exposed to chronic hypoxia grow slower and consume less oxygen. A previous study by Ninness et al. (2006) found a small increase in lactate in the tissues of hypoxia-exposed rainbow trout embryos just before hatch. Therefore, it is possible that embryos could increase their reliance on anaerobic metabolism during hypoxic stress, but the small change in lactate levels indicates that the overall anaerobic capacity is low and could only play a very minor role at these early life stages.

Two interesting findings that emerge from the 22 and 29 dpf data sets are that mass-specific M_{O_2} (*i*) is depressed by no more or less than 40% and (*ii*) decreases within 30 min of hypoxia exposure and not any further after 1 or 2 weeks of hypoxia. These results indicate that trout embryos are O_2 conformers, at least when exposed to 50% O_2 saturation between the eyed stage and hatching, similar to

Fig. 5. DO concentrations of the boundary layer in rainbow trout (*Oncorhynchus mykiss*) embryos 28 days post fertilization (dpf) exposed to two water flow rates in normoxia: 3 (shaded squares) and 6 mL·min^{-1} (solid circles) (10 °C). The boundary layer curve at 3 mL·min^{-1} was significantly lower than at 6 mL·min^{-1} ($F_{(1,156)}$, $p < 0.01$). The vertical broken line indicates the chorion surface. An asterisk indicates a significant difference in DO between embryos exposed to 3 and 6 mL·min^{-1} ($p < 0.05$). Values are means ± SE of the mean ($n = 7$).



Atlantic salmon (*Salmo salar*) embryos (Hamor and Garside 1979) and the classic principles of Fry (1957). The extent of metabolic depression may be dependent on the magnitude of the hypoxia; however, to our knowledge, there are no reports in the literature of M_{O_2} at O_2 levels less than 50% saturation. Ciuhandu et al. (2007) found that DO levels in the boundary layer outside the chorion in 30 dpf trout embryos exposed to 35% saturation increased between 30 min and 8 h of exposure, presumably because of a progressive metabolic depression.

The 40% decrease in metabolic rate in hypoxia in the present study would help to balance the O_2 demand to the reduced O_2 supply but still enable the embryo to grow (body mass increased by 10-fold between 15 and 29 dpf). Growth retardation and delayed development in salmonid embryos in response to hypoxic water are well established in the literature (e.g., Shumway et al. 1964; Hamor and Garside 1976; Ciuhandu et al. 2005). In the present study, we evaluated growth by measuring the dry mass of the embryos. As we did not "stage" or examine the morphology of the individual embryos, it is not possible to say how hypoxia exposure affected the development of these embryos. It is obvious, however, that the hypoxia exposure did negatively influence the growth of the embryos. It is also not known if the embryos that displayed a delay in growth and (or) development would be able to "catch up" if they were then exposed to normoxic water.

We hypothesized that water flow rate would affect the DO inside the chorion and in the boundary layer outside the chorion. Indeed, a decrease in water flow rate lowered the DO concentration inside the chorion and in the boundary layer to a magnitude similar to that of mild hypoxia. Furthermore, hypoxia and water flow rate may become additive, further decreasing DO. A reduction in water flow rate decreases the rate at which the boundary layer is renewed and results in low DO levels in the boundary layer (Ciuhandu et al. 2007). Also, the gradient of O_2 depletion from the free

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stream to the chorion and the significant drop in DO between the chorion surface and perivitelline fluid are evidence that the boundary layer and the chorion are limiting O_2 uptake (Pinder and Feder 1990; Ciuhandu et al. 2007).

Although flow rates are difficult to measure in natural salmonid redds, Zimmermann and Lapointe (2005) determined that in some redds, water flow is relatively slow, varying between 2.7 and 4.5 mL·min^{-1}. In the present study, slow flow rates resulted in unfavourable DO gradients, demonstrating the importance of water exchange even in highly saturated water. In the spawning redd, the embryos are covered with substrate for protection and the DO and velocity of the water are related to substrate size and permeability (Peters 1962).

By initiating the hypoxia exposure at the eyed stage, we studied the ability of animals to deal with low O_2 levels with a functioning circulation system. If we had started hypoxia prior to this time, there would likely have been differences between treatment groups in circulatory system development and effectiveness. These factors would further complicate the interpretation of our data. In addition, it is also expected that an earlier exposure of the embryos to hypoxia would also have had a greater effect on the metabolic processes of these animals. Future studies will build on the current study and characterize how hypoxia exposure, at an earlier developmental time, alters embryo O_2 consumption.

In conclusion, the O_2 concentration of the perivitelline fluid of rainbow trout embryos is significantly decreased by exposure to mild hypoxia. We showed for the first time that rainbow trout embryos exhibit a decrease in M_{O_2} during mild acute and chronic hypoxia within 2 weeks of hatching. This indicates that rainbow trout embryos are O_2 conformers and are capable of responding to acute hypoxia early in development by maintaining a balance in their aerobic energy. However, when O_2 supply becomes limiting at accelerated phases of embryonic growth, mass is reduced accordingly. The results also suggest that low water flow rates produce unfavourable boundary layer conditions and ultimately create a hypoxic microenvironment. Overall, the differences in responses to chronic or acute hypoxia exposure may reflect the fact that thresholds for physiological responses are defined by developmental stage and duration of the stimulus.

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Observations of avoidance reactions of migrating salmon to a mobile survey vessel in a riverine environment

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Abstract: Detailed avoidance reactions of adult migrating salmon to a mobile survey vessel were successfully observed with side-looking dual-frequency identification sonar (DIDSON) in the lower Fraser River (British Columbia, Canada). Both adult sockeye (*Oncorhynchus nerka*) and pink salmon (*Oncorhynchus gorbuscha*) returning to the river were found to avoid the approaching vessel by initiating lateral movements away from the vessel, making the fish unlikely to be insonified by the downward-looking transducers towed by the vessel. The vessel was found to have an estimated mean interference range of 4 m from its propeller. Analyses of the data concluded that once the vessel and fish were separated by more than 7 m, the vessel no longer affected the normal migration behaviour of the fish.

Résumé : Un sonar latéral d'identification à double fréquence (DIDSON) nous a permis d'observer avec succès les réactions détaillées d'évitement des saumons adultes en migration face à un navire mobile d'inventaire dans le cours inférieur du Fraser (Colombie-Britannique, Canada). Tant les saumons rouges (*Oncorhynchus nerka*) que les saumons roses (*Oncorhynchus gorbuscha*) qui retournent dans la rivière évitent le navire qui s'approche en entreprenant des déplacements latéraux pour se distancer du navire, ce qui fait que les poissons risquent peu d'être repérés par les capteurs sonar orientés vers le bas et tirés par le navire. La portée moyenne de l'interférence du navire est estimée à 4 m depuis l'hélice. Les analyses de ces données mènent à la conclusion que lorsque le navire et les poissons sont séparés par une distance de plus de 7 m, le navire n'affecte plus le comportement normal de migration des poissons.

[Traduit par la Rédaction]

Introduction

Vessel-based mobile acoustic survey is traditionally an effective sampling method for assessing abundance of fish stocks distributed over large areas in oceans, rivers, and lakes (McLennan and Simmonds 1992). Conventional fisheries sonar systems project sonic pulses at a central frequency ranging from 38 to 420 kHz to survey fish. The source levels of the sonar are normally set to 210–240 dB (ref. 1 μPa @ 1 m) (Urick 1983). The sounds produced by these pulses have been shown to be noninvasive to adult anadromous fish such as salmonids (Knudsen et al. 1992). However, engine and propeller noise from a survey vessel can disrupt normal swimming behaviour and natural distributions of fish, which may bias the survey results (Mitson 1995). Potential avoidance behaviour by fish is a major concern in vessel-based acoustic surveys. Addressing this concern requires delicate observations and measurements of behavioural reactions of fish to the presence of survey vessels in a natural environment where mobile surveys are carried out.

A substantial amount of research has been conducted to assess avoidance behaviour of herring to survey vessels at sea (Vabo et al. 2002; Mitson and Knudsen 2003). Based on a review of published studies on hearing mechanisms of several commercial fish species, Mitson (1995) reported that herring were most sensitive to sounds from 20 to 1200 Hz. This frequency band is well below the frequencies employed by most fisheries sonar. Mitson and Knudsen (2003) found that the radiated noise from the fisheries research vessel (FRV) *Johan Hjort* caused avoidance behaviour of herring schools at a distance of 540 m when the propeller shaft was operated at a speed of 125 rpm. After investigating the avoidance effect on acoustic surveys of wintering Norwegian spring-spawning herring, Vabo et al. (2002) concluded that FRV *Johan Hjort* estimated only 59% of the biomass. On the other hand, Fernandes et al. (2000) found that herring did not avoid FRV *Scotia*, a quiet ocean-going survey vessel that was built with a speed-related noise level below the threshold of $130 - 22 \log_{10} df$ kHz) dB (ref. 1 μPa·Hz^{-1} @ 1 m) in the frequency range between 1 and 100 kHz. This threshold was proposed as a guide for all fisheries research vessels in 1995 by the International Council for the Exploration of the Sea (ICES) (www.ices.dk/pubs/crr/crr209/crr209.pdf).

Despite the wide use of acoustic mobile surveys of fish

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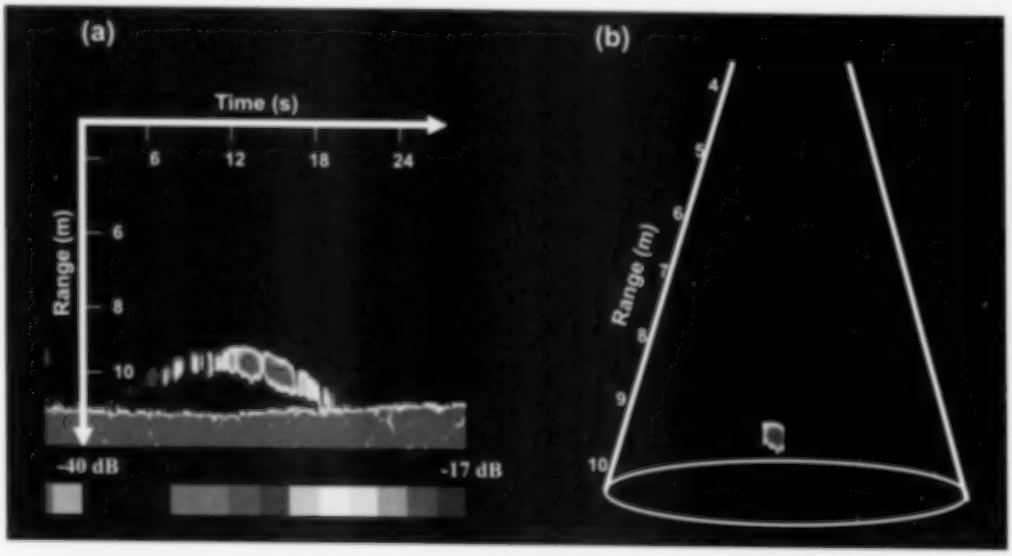
Fig. 1. (*a*) 20 s long echogram of a bottom-oriented target moving through a sound-beam projected by a 30° half-power full beam-width single-beam transducer driven by a Biosonics Model 104 sounder (www.biosonicsinc.com). Colours represent echo intensities. (*b*) Two seconds of echo intensities of the target (from 30 projected acoustic pings) as it reaches the closest point to the transducer. The signal-to-noise ratio during data collection was approximately 20 dB. Note that all referred transducers' beam widths hereafter use the half-power full beam width as their definitions.



stocks in rivers and lakes, there have been few reports on behavioural reactions of fish to survey vessels in these confined and shallow water environments. In assessing major sources of biases in the estimation of adult salmon abundance with a mobile survey system in the lower Fraser River (British Columbia, Canada), investigators hypothesized that avoidance behaviour is a source of negative bias (Mission Hydroacoustic Facility Working Group 1994; Xie et al. 2002). A recent study in the same area of the river indicated that the mobile survey system estimated only 36% of near-shore fish passages that were simultaneously monitored by a shore-based, side-looking split-beam sonar system (Xie et al. 2007). Collecting direct evidence of avoidance behaviour in a turbid river is a highly challenging task, as observations of behavioural reactions of fish to a survey vessel are difficult to obtain with single- or split-beam sonar systems. The difficulty is due to the uncertainty in recognizing fish from acquired echoes that are blended with noise from air bubbles and ship wakes, as demonstrated by a split-beam study on fish responses to the mobile survey vessel (Xie et al. 1997). Observations from that study and a subsequent investigation using an active tracking sonar system indicated that there were noticeable changes in the swimming direction of detected fish targets as the vessel approached. However, the data were not adequate to provide detailed and conclusive information on behavioural reactions of fish to the vessel (Cronkite et al. 2000; Hedgepeth et al. 2000). Efforts in these two studies were hampered by target-recognition uncertainty even when the signal-to-noise ratio was favourably high, let alone when noise from the engine and ship wakes overwhelmed echoes from fish as the vessel approached the monitoring area.

Target information acquired from split-beam sonar provides estimations on the target strength of detected fish and their point locations within the sound beam, which allow the sonar to measure swimming velocities of the fish from a series of tracked locations by consecutive acoustic pings (Ehrenberg and Torkelson 1996). A split-beam sonar system can achieve a range resolution down to a few centimetres by using submillisecond pulses. However, the system usually has a very coarse spatial resolution in the cross-range direction, as this resolution is limited by the half-power full beam width of the transducer. When the sound beam detects multiple targets from the same range, the sonar cannot resolve angular positions of these targets in the beam, as demonstrated by Cronkite and Enzenhofer (2002). For individual targets with lateral sizes comparable with the width of the sound beam at the range of detection, the sonar can only estimate point locations of the targets for each transmitted pulse with little information on the detailed geometric shapes of these targets. An echogram of a large target is shown as it moves through the sound beam projected by a downward-looking 30° half-power full beam-width transducer from a stationary vessel (Fig. 1*a*). Two seconds of echo intensities are then shown from this target as it reaches the closest point to the transducer (Fig. 1*b*). One can speculate that this is a large target but cannot positively identify the nature of the target from the acquired echo intensity and location data. The target was later identified as a Fraser River white sturgeon (*Acipenser transmontanus*) by an imaging sonar that simultaneously insonified the target from the same vessel (Fig. 2). Figures 1 and 2 are presented here to illustrate from a user's perspective that conventional sonar are not as effective as imaging sonar in differentiating fish from non-fish targets. The uncertainty in identifying targets hampers the ability of conventional sonar to ascertain

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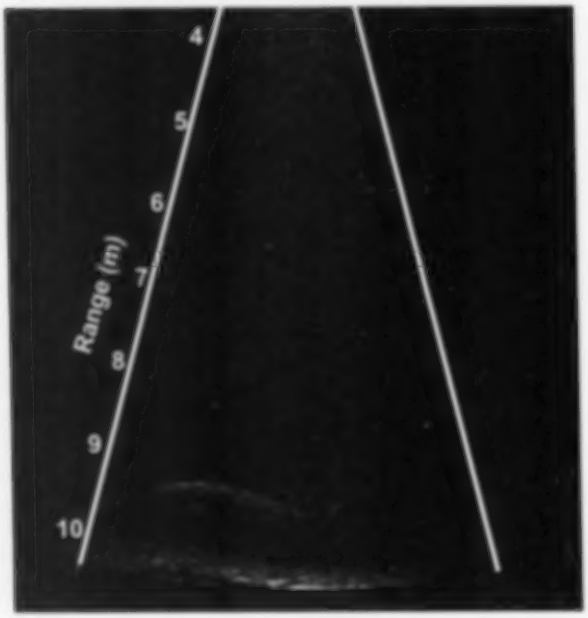
Fig. 2. Acoustic image of a Fraser River white sturgeon (*Acipenser transmontanus*) acquired with a downward-oriented standard dual-frequency identification sonar (DIDSON) operating at 1.8 MHz. The sonar was deployed from the port side of the survey vessel. Data were collected at a frame rate of eight frames per second over a 9 m long range window starting at 1.5 m. Morphologic features of the animal are visible from the image, including its fins, head, and tail. Also visible is the acoustic shadow cast by the fish on the river bottom. The length of the fish is estimated to be 2.1 m. This is the same fish detected by the single-beam transducer shown in Fig. 1. To view a DIDSON movie clip of the fish, go to www.soundmetrics.com/FM/fm.html.



behavioural reactions of fish when disturbed by vessels or predators.

To acquire morphological features of a fish, a sonar system should both insonify the entire fish with a wide sound beam and sonically illuminate the fish with adequate fine spatial details. This requires the transducer to produce a large field of view (FOV) with a sharp spatial resolution. The dual-frequency identification sonar (DIDSON) developed by Belcher et al. (2002) for fisheries applications achieves this requirement by forming a sound beam of 14° × 29° from up to 96 fan-shaped beams of 0.3° × 14° using a line-focused acoustic lens. The 29° FOV is practically wide enough to image fish in rivers and lakes (Moursund et al. 2003; Maxwell and Gove 2007). A standard DIDSON can operate in either the 1.8 MHz identification mode or the 1.1 MHz detection mode. These two operating modes result in two respective cross-range angular resolutions of 0.3° (1.8 MHz) and 0.6° (1.1 MHz) (see specifications at www.soundmetrics.com). A single frame of acoustic reflections from an insonified target forms an acoustic image of the target with an adequate cross-range resolution for its visual identification, as demonstrated by the sturgeon image (Fig. 2). In comparison with target information from conventional sonar, DIDSON images are visually intuitive, allowing users to identify an imaged target from its geometric shape. DIDSON can also operate at a fast sampling rate of up to 21 frames per second depending upon the selected length of the range window. This allows for image-tracking of moving targets in the FOV. Taking images at fast sampling rates captures the dynamics of moving targets, which is especially useful and effective in distinguishing fish from non-fish targets. The unique ability to identify fish among other underwater objects makes DIDSON an effective hydroacoustic tool for studying various aspects of fish behaviour. By alleviating target-recognition uncertainty, DIDSON can also provide more accurate estimates of migratory fish abundance in small streams or river channels where migrations are confined to nearshore areas (Holmes et al. 2005).

In this paper, we present data obtained with a DIDSON imaging sonar system in an experimental study on behavioural reactions of migratory adult salmon to a survey vessel in the lower Fraser River. The analysis of the data leads to an estimate of the interference range of the vessel.

Materials and methods

Acoustic image data were taken with a standard DIDSON unit between 30 August and 1 September 2005 in the Fraser River near Mission, British Columbia, when large numbers of pink salmon (*Oncorhynchus gorbuscha*) and sockeye salmon (*Oncorhynchus nerka*) co-migrated upstream past the Pacific Salmon Commission (PSC) Mission echosounding site. Using data acquired from the transducers towed by a mobile survey vessel and a shore-based split-beam sonar system (Banneheka et al. 1995; Xie et al. 2005), the PSC estimated that a total of 1.7 million sockeye and pink salmon passed the site over the 3-day period, which translated to a mean hourly passage rate of 23 600 fish·h^{-1}. A photo showing several key features of the estimation system at the site is provided (Fig. 3). A two-dimensional (2D) Cartesian frame originating from the location of the piling structure (49°08.175′N, 122°16.466′W) was chosen for positioning all horizontal variables in the study with the cross-river axis pointing at the bearing of 328° (Fig. 4), whereas the mean water height over the study time period was used as the reference for positioning all vertical variables.

The DIDSON unit was deployed on a steel tripod in the shallow water of the south bank of the river with the tripod being anchored on the river bottom. The sonar was aimed horizontally with the 29° FOV sampling an offshore area through which the transecting vessel consistently navigated. To maximize the sonar's FOV to monitor fish moving through the area, the 1.1 MHz detection mode was used to acquire image data with a 20 m long range window starting at 10 m and ending at 30 m. We visually verified that fish of adult salmon sizes could readily be identified in the acoustic images acquired with this setting. Also shown is a normal transect path recorded by a differential global positioning system (DGPS) on board the vessel as it moved through the monitoring area (Fig. 4).

Site characteristics and the typical fish distribution

The lower Fraser River at Mission is under tidal influen-

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Fig. 3. Photo taken from the south bank of the Pacific Salmon Commission (PSC) Mission echosounding site. Key features are labeled with letters *a*–*d*. (*a*) The mobile survey vessel with an inset photo of a close-up view; (*b*) a fixed piling structure, the location of which was chosen as the origin of the horizontal reference frame in the study; (*c*) a 25 m long deflection fence forcing adult salmon to swim through the field of view of the side-looking split-beam transducers; (*d*) deployment location of the south bank split-beam transducers (about 15 m upstream from the piling). The deployment location (outside the photo) of the dual-frequency identification sonar (DIDSON) was approximately 45 m downstream from the piling.

ces, which can cause a daily change in water height of up to 1.5 m in late August or early September when river discharge is typically low. The river's width can reach 420 m during high discharge periods in the spring. The river is turbid with a near-zero visibility, and visual detection of fish at the site is not possible. Salmon prefer to migrate upstream in nearshore waters where currents are slower (Xie et al. 2005) to minimize the amount of energy expended for the upriver journey (Hinch and Rand 1998). The bottom profile of the river is shown, as well as a cross-river fish distribution detected on 30 August by a 30° Biosonics single-beam transducer (www.biosonicsinc.com) towed by the survey vessel (Fig. 5). Fish migrating in the inshore area of up to 60 m from the south bank were also monitored by a 200 kHz HTI model 243 split-beam sonar system (www.htisonar.com), which was installed 50 m upstream from the DIDSON. This side-looking sonar monitored and estimated south bank fish flux and swimming behaviour after the fish had moved well beyond the transect paths of the vessel (Fig. 4), thus providing estimations of abundance and behaviour of the fish migrating in the nearshore area free of potential interference from the vessel.

Main characteristics of the vessel

The vessel was a 6.7 m long aluminum hull motorboat manufactured by Raider Aluminum Boats in Surrey, British Columbia, in the late 1980s. The width of the vessel's beam was 2.6 m, and it had a 0.9 m draft in fresh water. The moderately V-shaped hull was designed specifically for surveying fish in shallow waters. The vessel was powered with a stern-drive Volvo Penta 3.0 L gasoline engine, which drove a three-bladed propeller. A global positioning system (GPS) receiver was installed onboard to record the transecting trajectory and speed during mobile surveys. A Biosonics 30° single-beam and a HTI 15° split-beam transducers were deployed from the vessel to survey fish across the river. The two transducers were mounted on two towed bodies on each side of the vessel and were kept in a downward-looking orientation while being towed. During normal transecting, the propeller was kept at a rotational speed ranging from 1200 to 2000 r·min^{-1} (1 r = 2π rad) depending upon the discharge level and tidal currents. On average, it took about 5 min for the vessel to complete a cross-river transect at a mean cruising speed of 1.4 m·s^{-1} (2.7 kn; 1 kn = 1.852 km·h^{-1}). This was a quiet vessel in comparison with other vessels and pleasure boats frequently navigating the local water and was much smaller than the 68 m long FRV *Scotia*, capable of transecting the sea at a speed of 11 kn (Mitson and Knudsen 2003).

Experimental setups

The horizontal distance between the vessel and the DIDSON location was gauged and recorded by the GPS receiver. The DIDSON unit continuously collected image data over the time period between 1500 on 30 August and 0000 on 1 September. The plan view of the monitoring area is shown (Fig. 4). The sonar's wide-beam axis was aimed at an angle of –6° relative to the horizon to obtain an optimal fit of the

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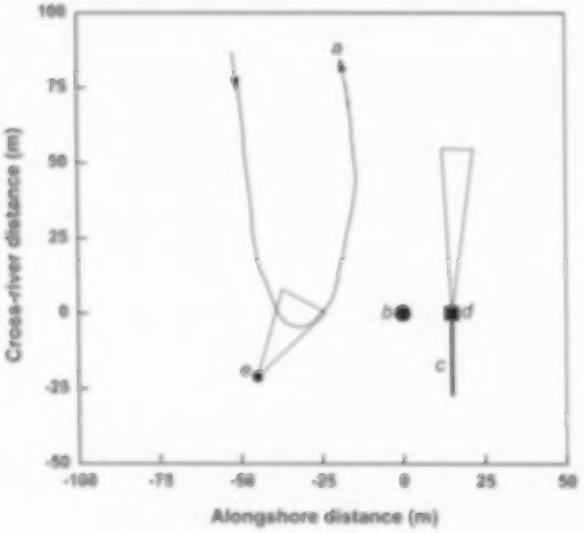
Fig. 4. A global positioning system (GPS)-gauged transect path of the vessel as it approached and departed the south bank monitoring areas on 30 August 2005. (*a*) Transect trajectory of the vessel; (*b*) location of a fixed piling structure chosen as the origin of the horizontal reference frame; (*c*) a 25 m long deflection fence forcing adult salmon to swim through the field of view (FOV) of the side-looking split-beam transducers; (*d*) location of the south bank split-beam transducers; (*e*) the deployment location of the dual-frequency identification sonar (DIDSON). The two triangles outline the FOV of the two sonar systems.



wide beam to the water column. To maximize the likelihood of observing behavioural reactions of fish to the survey vessel, the sonar beam was aimed at the turning area of the transect path where the vessel approached the south bank and altered its course towards the north bank. With a mean cruising speed of 1.4 m·s^{-1}, the vessel repeatedly transected the monitoring area at a typical time interval of 10 min, which included two 5 min transects to and from the north shore of the river, thus providing multiple opportunities to observe fish–vessel interactions.

Identification of abnormal behaviour

The identification of avoidance responses of migrating fish to the survey vessel was based on the knowledge of normal swimming behaviour of the fish free of vessel interference. To assess normal migration behaviour, speed and direction of travel of several thousand imaged fish were examined when the vessel was beyond 100 m from the FOV of the DIDSON. Also included in the study of normal swimming behaviour were more than 10 000 fish targets detected by the south bank split-beam sonar system (Figs. 4 and 6). The analysis of these undisturbed fish data showed that the direction of travel was an effective variable to separate normal and abnormal swimming behaviour. Direction of travel of an imaged fish is determined by the cross-range swimming direction as the fish exits the FOV. If the fish exits the FOV in a direction that is consistent with the general upstream-swimming direction displayed by the thousands of

Fig. 5. Cross-river fish distribution detected by the mobile survey vessel on 30 August 2005 at the Pacific Salmon Commission echo-sounding site. Each dot represents the depth and distance of a fish target estimated by the downward looking 30° Biosonics single-beam transducer and a GPS receiver. Fish migrating in the inshore area of the thick vertical line were also monitored and enumerated by the south bank split-beam sonar system. The horizontal arrow indicates an inferred suitable mobile survey area.

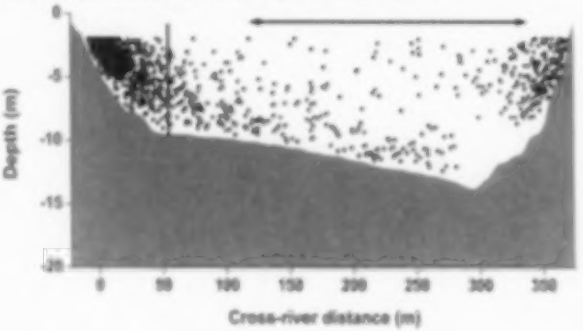
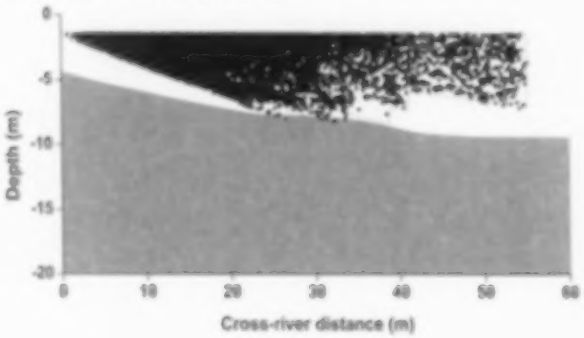


Fig. 6. Fish distribution observed by a side-looking 2° × 10° south bank split-beam transducer over a 16 h time period between 1300 on 30 August and 0400 on 31 August 2005. The transducer was sequentially aimed at seven vertical angles of –1°, –3°, –5°, –7°, –9°, –11°, and –13° relative to the horizon to sample the water column with a systematic hourly sampling effort of 6 min·aim^{-1}. Each dot represents the depth and distance of a single fish target. A total of 11 951 fish targets were detected by the transducer over this time period. The gray lines outline the coverage area of the nearshore water by the transducer's seven vertical aims.



undisturbed fish observed by the sonar systems, the fish is considered migrating upstream.

Categories of observed events

Since observations of changes in the swimming behaviour of fish were limited to the FOV of the DIDSON, we classified observed events into three categories as follows: (I) The initial avoidance reaction was captured by the sonar as the vessel approached the fish. (II) Avoidance reaction was clearly displayed on the acoustic image as the vessel approached the fish, but the sonar did not capture the timing of the onset of the avoidance, as the initial reaction occurred outside the FOV. Fish, affected by the reacting fish by joining their abnormal movements, were also included in this

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category. (III) The sonar observed no reaction behaviour as the vessel approached and departed the monitored area.

Category I events provide valuable information for a quantitative assessment of the interference range of the vessel. Category II events support the hypothesis that fish do react to the survey vessel, though the information from these events is inadequate for assessing the interference range of the vessel. Category III events can be used to assess interference-free ranges between the fish and the vessel. All reacting fish observed in this study reacted to the intrusion of the vessel by moving away from it. No attraction behaviour was observed.

DIDSON-estimated apparent spacing versus true spacing between targets

As a 2D sonar, the DIDSON could not position fish on the 14° beam plane, which coincided with the vertical plane in this study. Therefore, the vertical spacing between the vessel and the reacting fish could not be estimated from the image. The apparent spacing between a surface vessel and a bottom-oriented fish in the DIDSON's FOV underestimates their true 3D spacing (schematically illustrated in Fig. 7). Using variable notations of Fig. 7*a*, the true spacing can be expressed as

$$(1) \qquad \Delta R = \sqrt{|\boldsymbol{R}_1|^2 + |\boldsymbol{R}_2|^2 - 2 \cdot |\boldsymbol{R}_1| \cdot |\boldsymbol{R}_2| \cdot \cos(\Delta\Omega)}$$

where $\boldsymbol{R}_1$ and $\boldsymbol{R}_2$ are 3D vectors from the DIDSON to the vessel and fish, respectively, $\Delta\Omega$ is the angle between $\boldsymbol{R}_1$ and $\boldsymbol{R}_2$, and ΔR is the 3D distance between the vessel and the fish.

Upon being transformed onto the 2D FOV plane, the coordinates of the vessel and the fish are estimated from the DIDSON as $(|\boldsymbol{R}_1|, \theta_1)$ and $(|\boldsymbol{R}_2|, \theta_2)$, respectively (Fig. 7*b*). This transformation preserves the magnitudes of $\boldsymbol{R}_1$ and $\boldsymbol{R}_2$ by acoustically ranging the two targets through the sound speed and travel times, but it underestimates $\Delta\Omega$, the angle between the two vectors. This is because the apparent angle $\Delta\theta$ between $\boldsymbol{R}_1$ and $\boldsymbol{R}_2$ on the FOV plane is less than $\Delta\Omega$, owing to the lack of spatial resolution on the 14° beam plane. The apparent spacing estimated from the DIDSON is

$$(2) \qquad \Delta L = \sqrt{|\boldsymbol{R}_1|^2 + |\boldsymbol{R}_2|^2 - 2 \cdot |\boldsymbol{R}_1| \cdot |\boldsymbol{R}_2| \cdot \cos(\Delta\theta)}$$

Since $\Delta\theta \leq \Delta\Omega$, according to eqs. 1 and 2, $\Delta L \leq \Delta R$. Therefore, the DIDSON underestimates the true spacing. The only case in which the apparent spacing is equal to the true spacing is when $\boldsymbol{R}_1$ and $\boldsymbol{R}_2$ possess an identical angle with the acoustic axes on their respective 14° beam planes so that $\Delta\theta = \Delta\Omega$. For convenience, we will hereafter treat the apparent spacing ΔL defined by eq. 2 as "true" horizontal spacing whenever it is used for measuring distances between the vessel and fish in the FOV of the DIDSON. It should be noted that unless the two vectors lie on the horizontal plane, $|\boldsymbol{R}_1|$ and $|\boldsymbol{R}_2|$ are always greater than their corresponding horizontal components, which, if available from other measurements, should have been used in eq. 2 for precise calculations of the horizontal spacing between $\boldsymbol{R}_1$ and $\boldsymbol{R}_2$.

Estimation of the horizontal interference range of the vessel

In this study, we estimate the horizontal interference range by taking measurements of ΔL between the vessel and the reacting fish from Category I events. Evaluation of the horizontal interference range of the vessel using Category I events ensures that (*i*) ranges and azimuthal angles of the observed fish can be precisely positioned by the sonar on the 2D frame at the times of their initial reactions, and (*ii*) observed behavioural reactions most likely result from

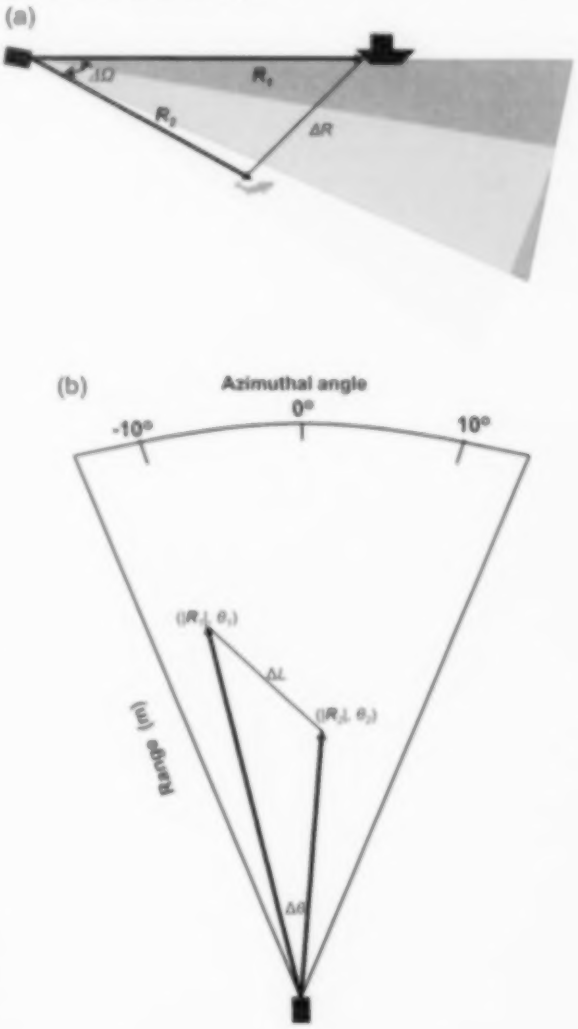

Fig. 7. Apparent spacing versus the true spacing between a surface vessel and a fish near the bottom. (*a*) Locations of the vessel and the fish on the 14° vertical beam planes of two individual beams outlined by the two triangles of the light and dark shades. $\boldsymbol{R}_1$ and $\boldsymbol{R}_2$ are three-dimensional (3D) vectors from the dual-frequency identification sonar (DIDSON) to the vessel and fish, respectively. $\Delta\Omega$ is the angle between $\boldsymbol{R}_1$ and $\boldsymbol{R}_2$, and ΔR is the 3D distance between the vessel and the fish. (*b*) Apparent locations of the vessel and the fish on the DIDSON's 2D field of view (FOV) plane. The two locations are defined by the 2D DIDSON coordinates of range $|\boldsymbol{R}|$ and azimuthal angle θ. The apparent spacing between the vessel and the fish is ΔL in the FOV.

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intrusions of the vessel rather than from other species or predators such as sturgeon or harbour seals (*Phoca vitulina*), which are known to frequent the local water to prey on salmon.

For most Category I events, ΔL was measured directly from the corresponding acoustic images between the reacting fish and the boat wakes right behind the propeller of the vessel. For events in which the vessel was outside the FOV of the sonar, ΔL was estimated from vessel locations provided by the GPS at the times of fish reaction. Since most of the observed reacting fish migrated in schools, the position of the first reacting fish was used for estimating ΔL for the entire school, whereas for Category II and III events, the position of the lead fish was used for estimating the horizontal range to the vessel.

Estimation of the 3D interference range of the vessel

The 3D interference range S for individual observations can be approximately formulated as

$$S = \sqrt{\Delta L^2 + \Delta D^2} \quad (3)$$

where ΔL is the 2D interference range of the vessel, and ΔD is the depth of the reacting fish. Although there was no information on ΔD, the river depth at the location where the fish began reacting was known. Since the fish must be located somewhere between the surface and bottom of the river, we first examine two extreme cases where the reacting fish are assumed to be swimming near the surface ($\Delta D \approx 0$) or near the river bottom ($\Delta D \approx$ the water depth). These two cases lead to estimates of two nonparametric probability density functions for the minimum and maximum interference ranges, S_{min} and S_{max}, respectively. Second, we project the distribution of ΔD from the vertical distribution data acquired by the nearby south bank split-beam sonar system (Figs. 4 and 6). We approximate the true distribution of ΔD with this inferred distribution from the split-beam data. With the projected distribution of fish depth and observed horizontal interference ranges from Category I events, we perform a large number of Markov chain Monte Carlo (MCMC) simulations to estimate the distribution of S and its point estimates (Gelman et al. 1995).

Results

Normal migration behaviour

To assess the normal migration behaviour of fish in the absence of intrusions by the survey vessel, we examined the direction of travel of detected fish from the following two data sources.

Fish imaged by the DIDSON

A total of 5651 individual fish were identified from the DIDSON images when the vessel was more than 100 m away from the monitoring area. These fish were randomly sampled from 14 h of continuous recordings of the DIDSON data starting at 1500 on 30 August. Of the 5651 fish, only 43 were found moving downstream. The total cumulative time for the passage of these fish was 1.16 h. This corresponds to an estimated hourly upstream passage rate of 4834 fish·h^{-1}, or 21% of the total hourly passage rate of 23 600 fish·h^{-1} across the entire river.

Fish targets detected by the split-beam sonar

A total of 11 951 fish targets were observed by the south bank $2^{\circ} \times 10^{\circ}$ split-beam transducer when the fish had moved well beyond the transecting paths of the vessel (Figs. 4 and 6). These targets, with 183 going downstream, were detected by the transducer using a systematic hourly sampling effort of 6 min·h^{-1} starting at 1300 on 30 August for a total of 16 h. This leads to an estimated hourly upstream passage rate of 7355 fish·h^{-1} through the monitoring area by this transducer or 31% of the total hourly passage rate. The sampling design and data-processing procedures of the split-beam system are described in Xie et al. (2005).

Based on the direction of travel analysis of these two data sets, we found over 98% of the fish migrating upstream with the split-beam sonar indicating a mean upstream swim speed of 0.7 m·s^{-1}. We concluded that upstream migration was the natural fish behaviour during the study time period.

Evidence of avoidance behaviour

A total of 90 schools of fish (1662 individuals) were identified as events of interest for detailed analyses in the study. Each event started at the time when the vessel was estimated at a 100 m range from the DIDSON while approaching the south bank. Of the 90 events, 57 showed evident avoidance behaviour in reaction to the approaching vessel (Category I and II). The remaining 33 events (Category III) showed no reaction to the vessel. Images showing an example of a school of fish moving normally through the FOV when the vessel was about 33 m from the sonar are provided (Fig. 8). This school, composed of five individual fish, migrated upstream at a swimming speed of 0.77 m·s^{-1}. Also worth noting is the consistent formation of the fish as they moved through the FOV in a synchronized fashion. Reactions of another school of 10 upstream-swimming fish as the vessel approached are also shown (Fig. 9). The images captured sudden behavioural changes of the fish over a time interval of 7 s. These changes are summarized as follows: (*i*) At t_1, the vessel entered the FOV. All the fish in the school appeared to be swimming upstream normally up to this point of time. (*ii*) At t_2, the fish began reacting to the approaching vessel by deviating away from the vessel. The closest horizontal range between the fish and the vessel was 4 m before the fish started a lateral movement. (*iii*) At t_3, the fish completed the maximum lateral movement of 3 m. The closest horizontal range between the fish and the vessel was approximately 7 m. With the departure of the vessel, the fish appeared to make a hard lateral turn to resume the normal upstream migration. (*iv*) The fish became scattered with individual fish exhibiting nonuniform swimming behaviour in reaction to intrusions of the vessel. This nonuniform behaviour among individuals in the school increased the apparent size of the school and decreased the tightness of the formation relative to that observed at t_1.

Estimation of the horizontal interference range of the vessel

Of the 57 fish schools, 25 were identified as Category I events for estimating the horizontal interference range of

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Fig. 8. Three overlaid dual-frequency identification sonar (DIDSON) images of a school of five undisturbed fish (inside the three ellipses) migrating upstream through the field of view of the DIDSON at a mean speed of 0.77 m·s^{-1}. The long arrow is the upstream-migrating direction of the fish. The time interval between the first and third images was 7 s. The approaching vessel (rectangular box) was 33 m away from the sonar. Colours represent relative echo intensities of the fish, with blue to red representing lower to higher intensity levels, respectively. For presentation clarity, static patterns from the river bottom are removed from these and subsequent images.

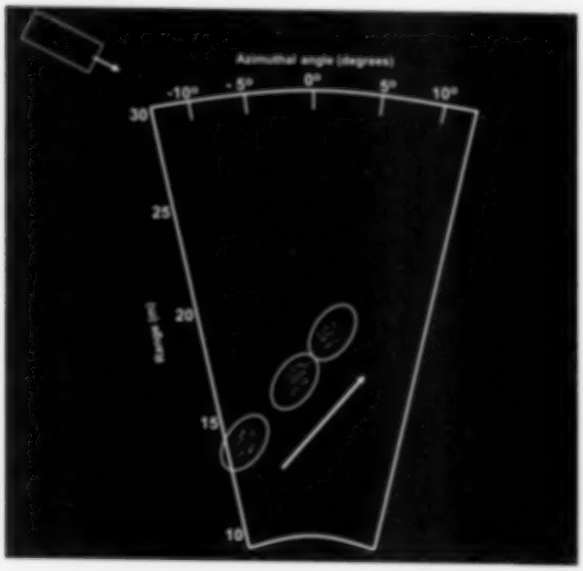
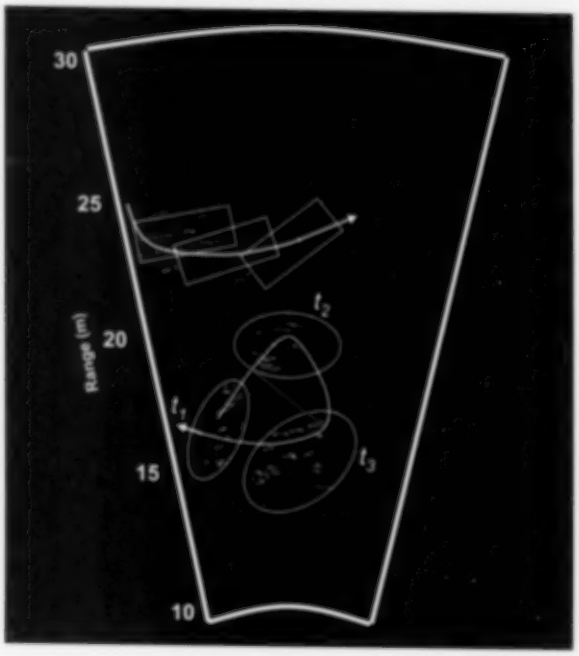


Fig. 9. Three overlaid dual-frequency identification sonar (DIDSON) images of a school of approximately 10 fish at times t_1, t_2, and t_3 over a 7 s time interval (i.e., $t_3 - t_1 = 7$ s). The rectangular boxes are the approximate locations of the vessel at the three instances, with a broken line showing a 10 s vessel trajectory. The vessel's hull is identifiable from the image at t_1, and the boat wake can be seen at t_2 and t_3. The curved arrow outlines a general moving trajectory of the fish over the 7 s interval, with three ellipses of different sizes indicating changes in the school formation when the fish exhibited avoidance behaviour. The green arrow is the 3 m maximum lateral movement of the fish.



the vessel. These 25 schools comprised a total of 313 individuals. The measured ΔL values are tabulated together with the water depth and the range from the sonar for each of the 25 reacting fish (Table 1). A probability density function (PDF) for ΔL was estimated from these values and plotted (Fig. 10*a*). From the PDF, we obtained a mean of 3.8 m, a standard deviation of 1.5 m, and a 95% probability interval (PI) between 0.9 and 7.0 m. If all the reacting fish were migrating in the surface water, then the 3D interference range S reduces to the 2D range ΔL by setting $\Delta D = 0$ in eq. 3. Therefore, the ΔL corresponds to S_{min}.

Estimation of the 3D interference range of the vessel

Since the depths of the observed reacting fish were uncertain, we could only infer the 3D interference range by assuming a depth distribution. A total of three distributions were examined for modeling fish depth, which included two extreme cases and one general case.

Extreme cases: fish migrate near the bottom or at the surface

In the case of bottom-oriented migration, S reaches its maximum by setting $\Delta D = \Delta D_{max}$ in eq. 3. The water depths ΔD_{max} at the locations of the 25 Category I events are listed (Table 1). The estimated distribution of S_{max} is plotted (Fig. 11), which leads to a mean of 4.8 m, a standard deviation of 1.3 m, and a 95% PI between 2 and 7.4 m. The case of surface migration is discussed in the previous subsection, and the estimated distribution of S_{min} is plotted for visual comparisons among estimated density functions under different assumptions of fish depths (Fig. 11).

General case: fish follow a depth distribution

The two extreme depth distributions of fish being near the river boundaries help establish bounds for the 3D interference range, but they are unlikely to represent the true distribution of the fish. The best available data for estimating the true distribution of fish depth for this study came from the fish targets detected by the nearby south bank split-beam sonar (as shown in Fig. 6). A total of 2324 split-beam fish targets in the range bin between 20 and 30 m were chosen to estimate the depth distribution as the side-looking transducer sampled a large portion of the water column over this range bin with a minimum blind zone near the bottom area. Depths of individual targets were scaled relative to the maximum water depth within the range bin. As a result, target depths were transformed to a standardized vertical scale ranging from 0 to 1, with 0 being the surface and 1 being the bottom of the river. The vertical distributions of the split-beam targets with an estimated median depth at 0.4 are

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Table 1. Observed horizontal interference ranges of the survey vessel to the 25 schools of fish (Category I events).

Event time	Estimated horizontal range, ΔL (m)	Water depth, ΔD_{max} (m)	Range to DIDSON (m)	Estimated lateral movement (m)	Estimated no. of fish in school
30 August					
15:19:17	2.9	3.7	21.5	2.8	16
15:29:54	8.0	3.6	20.5	4.5	17
15:39:55	3.0	3.2	17.9	1.9	8
17:50:03	4.7	2.9	16.2	2.6	8
18:00:23	2.9	3.5	19.9	5.4	7
18:09:57	3.1	3.0	16.9	4.0	31
18:29:40	5.6	2.7	14.8	3.3	15
19:24:52	3.4	2.6	14.2	0.7	17
19:45:00	4.3	2.1	11.1	2.2	8
20:04:35	2.0	2.4	12.9	1.4	2
20:14:40	3.3	2.8	15.6	2.6	6
22:10:21	2.7	3.2	18.2	2.2	8
22:20:06	4.1	3.2	18.1	6.0	10
22:30:05	3.9	3.0	17.1	4.4	3
23:30:03	2.0	2.3	12.2	4.1	8
31 August					
03:45:30	2.7	2.7	15.2	2.4	11
15:40:05	3.2	3.1	17.3	5.8	16
16:09:52	2.9	3.0	16.8	2.0	27
16:20:01	6.2	2.8	15.3	1.4	12
17:34:18	3.8	3.0	16.7	0.9	13
17:45:17	4.7	2.8	15.3	2.2	20
18:15:13	4.1	2.4	12.9	5.6	13
18:25:19	4.0	2.4	12.8	0.5	13
20:25:06	4.6	2.3	12.4	1.5	10
21:50:02	2.7	3.0	16.7	1.1	14

Note: Also listed are the water depth, the range from the dual-frequency identification sonar (DIDSON), the estimated lateral movement, and the estimated number of fish for each of the 25 schools. The 25 schools comprised a total of 313 individual fish.

shown (Fig. 12). The data indicate that there were more fish migrating in the upper water column than near the bottom. This is consistent with the unique behaviour of late-run sockeye as they respond to increasingly large tidal changes in late August when the tide switches from ebbing to flooding in the lower river near the site. We assume that the fish migrating through the DIDSON monitoring area follow a similar depth distribution as that of the split-beam fish targets on the standardized vertical scale. Under this assumption, the distribution in Fig. 12 is chosen as an approximate distribution for ΔD in eq. 3. We calculated the 3D interference range S for each of the 25 Category I events using eq. 3 by sampling the standardized depth from this distribution and multiplying the samples with the location-specific river depth to project ΔD for each of the 25 locations. Assuming S is log-normally distributed across all 25 Category I events, we estimated the mean and variance of the log-transformed 3D interference range (denoted as ln$S$) among all of these events and obtained a sample of ln$S$ from the inferred normal distribution. This sample of ln$S$ was then transformed back to the original arithmetic scale. The process was repeated 50 000 times using an MCMC simulation routine developed under WinBUGS 1.4 (Thomas et al. 1992). The 50 000 MCMC simulated S values led to an estimated distribution of the 3D interference range (as shown in Fig. 11). The resulting point estimates of S from this distribution include a mean of 4.1 m, a standard deviation of 1.2 m, and a 95% PI between 2.2 and 7 m.

Observed lateral avoidance movements of reacting fish

The lateral avoidance movement was estimated by the maximum perpendicular displacement that a reacting fish made relative to its normal swimming trajectory prior to the reaction, and this displacement is illustrated (Fig. 9). The estimated lateral movements of the first reacting fish under Category I are listed, which show a mean lateral avoidance movement of 2.9 m with a standard deviation of 1.7 m (Table 1).

Observed horizontal interference-free ranges from the vessel

The 33 Category III events provide observations of interference-free ranges from the vessel when the vessel was within 100 m of the DIDSON location. The separation distances between the fish and vessel for the 33 events are tabulated (Table 2). Category III events did not include data when the vessel was more than 100 m away from the DIDSON. As a result, the upper bound of listed values of horizontal interference-free ranges is noninformative (Table 2), whereas the lower bound of 8.7 m is consistent with the

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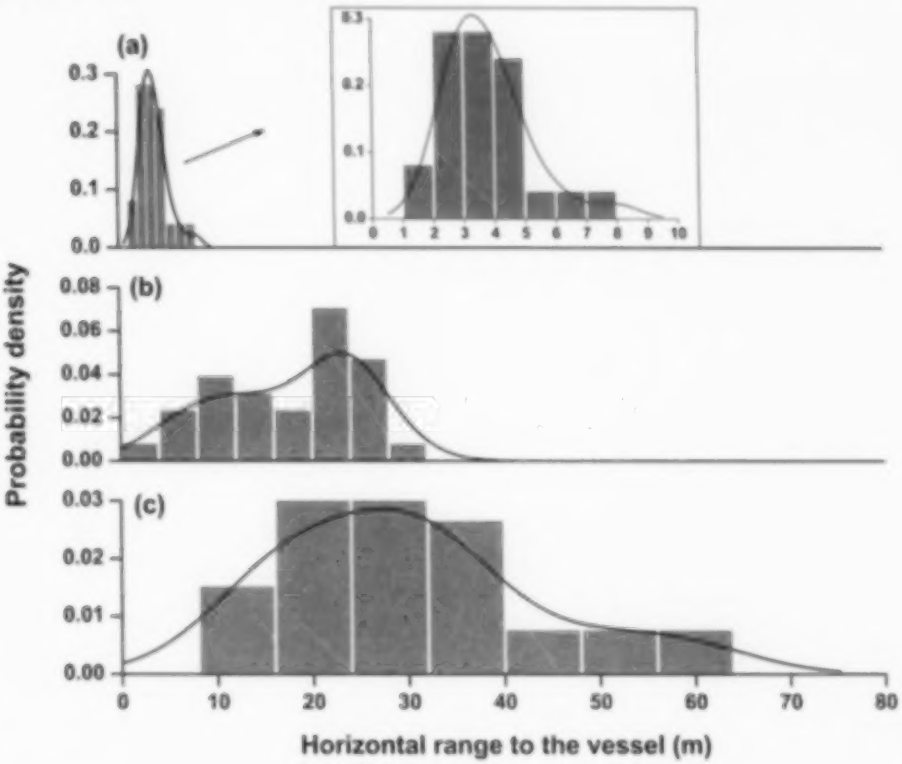
Fig. 10. Histograms (scaled as probability densities) of horizontal ranges from fish to the vessel. The line plots are estimated densities from the histogram data using an algorithm by Bowman and Azzalini (1997). Plots in the three panels are estimated densities from the 25 first-reacting fish from Category I (panel *a*), the 32 lead fish from Category II (panel *b*), and the 33 lead fish from Category III (panel *c*). The inset in panel (*a*) shows the probability density of the two-dimensional interference range of the vessel (estimated from Category I events) on a detailed scale. Note that the upper bound of the histogram in panel (*c*) is noninformative, as it is an artifact of the exclusion of events when the vessel was 100 m away.



upper bound of the 95% PI of 7 m of the 3D interference range. For comparisons, histograms of observed horizontal ranges for all the 90 events from Categories I, II, and III are plotted on a common *x* axis (Fig. 10).

Discussion

The results of this study show that migrating salmon in the river avoid the mobile survey vessel. The use of the DIDSON image sonar makes it possible to quantitatively assess the interference range of a survey vessel in shallow waters. All fish disturbed by the vessel were found exhibiting lateral movements to avoid the approaching vessel. The fish might have adopted lateral movement as an efficient way to quickly resume normal migration as the vessel departed. The magnitudes of observed lateral movements positioned the fish at least 2 m away from the vessel's transect path. This made it unlikely for the downward-looking transducers to detect reacting fish in shallow waters. Also worth noting are some Category II events that showed normal migrating fish were affected by avoidance behaviour of other schools of reacting fish by joining the downstream movement of the reacting fish. These events resulted in the second mode around 20 m in the distribution of the horizontal ranges.

Based on an inferred fish depth distribution obtained from the split-beam data, we predict a mean of 4 m and a 95% PI between 2.2 and 7 m for the 3D interference range of the vessel. These results indicate that the vessel will likely disturb adult migrating salmon if the spacing between the vessel and the fish is less than 4 m. Fish migrating in depths greater than 7 m are unlikely to be affected by the survey vessel even if the vessel passes directly above them. In the absence of interference from the vessel, we found that 24% and 31% of the total salmon migration occurred in the two respective monitoring areas by the two sonar systems near the south bank. With so many fish migrating in nearshore waters, the intrusion of the vessel can cause a large negative bias in the mobile survey results. While the vessel is not suitable for surveying fish in nearshore waters less than 4 m deep, it can be used to survey fish in deep offshore water if the majority of the offshore fish migrate at depths greater than 4 m. Assuming the offshore fish follow a similar vertical distribution to that detected by the south bank split-beam sonar with a median depth at 0.4 on the standardized vertical scale, we can project a profile of median fish depth

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Fig. 11. Estimated probability densities of the three-dimensional interference range of the vessel by assuming fish migrate in surface water (thin line), migrate near the bottom (broken line), or follow the same depth distribution as that of the fish detected by the south bank 2° × 10° split-beam transducer (thick line). Point estimates and 95% probability intervals derived from these density distributions are described in the corresponding text.

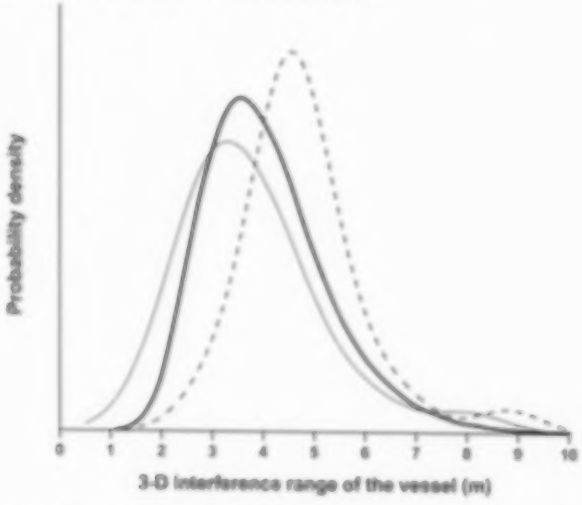
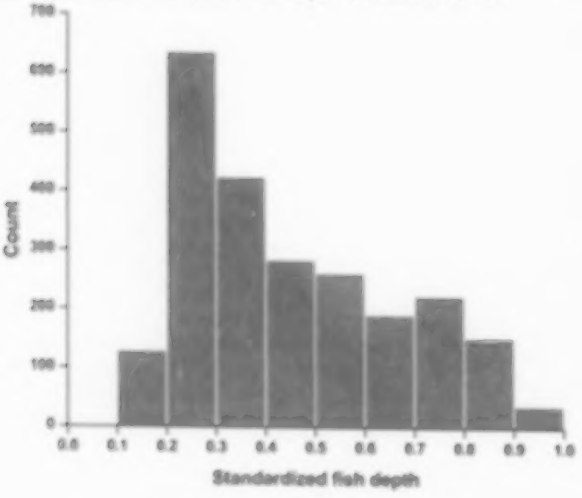


Fig. 12. Histogram of target depths of 2324 fish targets detected by the south bank 2° × 10° split-beam transducer in the range bin between 20 and 30 m. The data are scaled relative to the maximum river depth in the range bin, with 0 corresponding to the surface and 1 to the bottom. The median depth is estimated at 0.4.



across the river and locate the cross-river distances at which the profile intercepts the 4 m interference surface layer from the vessel. Using this method, we can statistically identify an offshore area between 120 and 338 m from the south bank as a suitable mobile survey area where the majority of the fish are projected to be present at depths greater than the mean interference range of 4 m. The size of this survey area is evidently controlled by the interference range of the vessel and the vertical distribution of the fish. For instance, in contrast with the behaviour of late-run sockeye, earlier returning stocks such as summer-run sockeye usually migrate near the river bottom (Xie et al. 2002), which would result in a lower profile of median fish depth than that of late-run sockeye. As the median fish depth increases or the interference range of the survey vessel decreases, the suitable mobile survey area will expand into nearshore waters. Nevertheless, the nearshore fish are better enumerated by nonintrusive, shore-based, side-looking sonar systems. A combined use of shore-based sonar and vessel-based mobile sounding systems can meet the demand of sampling fish in a large river while minimizing the impact of the vessel on fish migrating in nearshore waters. Such a sampling configuration has been implemented at the PSC Mission echosounding site since 2004 to aid in-season management of the

Table 2. Interference-free ranges between the fish and the approaching vessel estimated for 33 schools of fish (Category III events) comprising a total of 602 individual fish.

Event time	Estimated horizontal range, ΔL (m)	Maximum fish depth, ΔH_{max} (m)	Range to DIDSON (m)	Estimated no. of fish in school
30 August				
14:04:51	52.5	3.9	22.7	15
17:24:25	22.3	5.0	29.6	8
31 August				
01:20:21	34.8	3.9	22.8	10
05:39:43	38.3	4.3	24.8	14
05:49:36	34.5	3.3	18.9	22
06:09:36	20.6	3.7	21.0	12
06:19:30	15.4	3.3	18.4	16
06:29:10	33.2	3.8	22.1	12
06:39:25	15.1	3.3	18.6	27
07:24:28	26.6	3.2	18.3	28
07:34:24	28.9	2.9	16.1	21
07:54:54	8.7	3.9	22.6	39
08:05:07	43.2	3.5	19.8	12
08:14:40	17.0	3.3	18.7	18
08:24:38	14.4	3.8	21.9	11
09:29:44	28.5	3.3	18.9	30
09:39:46	26.8	3.8	22.1	23
10:09:35	16.5	3.1	17.7	30
10:19:30	61.9	3.1	17.7	23
10:29:29	22.2	3.4	19.2	23
10:39:24	17.7	3.3	18.6	16
11:19:45	18.8	3.8	21.7	35
11:29:48	29.5	3.8	21.7	28
11:49:42	34.0	3.3	19.0	32
12:19:40	46.8	3.0	17.1	43
12:29:29	16.7	3.4	19.3	22
19:24:10	59.4	2.5	13.5	7
19:34:02	53.9	2.6	14.3	6
20:04:43	32.7	2.5	13.3	7
20:14:14	30.1	2.6	14.3	2
22:09:48	26.1	3.3	19.0	4
22:19:55	31.9	3.4	19.4	3
23:29:38	33.3	2.7	14.9	3

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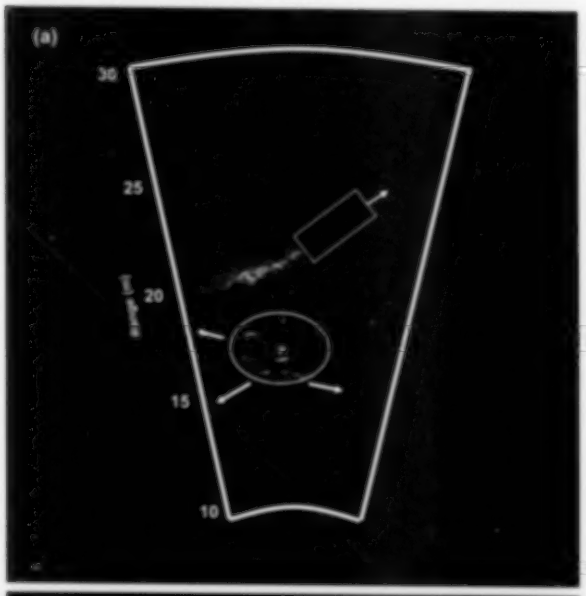
Fig. 13. (*a*) Image showing a school of eight initially upstream-migrating fish being scattered by the wake of the vessel (shown as a rectangle). The closest distance from the fish to the propeller is estimated at 4 m. The arrows indicate the main scattering directions of the fish. (*b*) Image taken 18 s later showing another school of nine fish swimming upstream at a normal speed of 0.78 m·s^{-1} despite a large cloud of air bubbles drifting downstream right above the fish at a speed of 0.28 m·s^{-1}. The bubbles resulted from the dissipating boat wake from the vessel, which was approximately 20 m away from the fish. The solid arrow indicates the swimming direction of the fish, and the broken arrow shows the downstream direction of the drifting cloud of bubbles.



Fraser River sockeye and pink salmon fisheries (Xie et al. 2005).

Fish migrating in the lower Fraser River are constantly subject to noise radiated from engines and propellers of various types of vessels that navigate the river. Our sonar observations indicate that this man-made noise field alone does not disrupt the overall upstream migration behaviour of salmon. However, we did observe from DIDSON images that fish displayed strong avoidance reactions when encountering boat wakes generated by the propeller. Strong reactions of a school of eight upstream-migrating fish encountering the boat wake of the vessel are shown (Fig. 13*a*). This was followed 18 s later by another school of nine upstream-migrating fish when a large cloud of air bubbles dissipated from the boat wake drifted downstream right above the fish (Fig. 13*b*). The fish did not appear to be affected by the surface-drifting air bubbles. This may indicate that a propeller running at a high rpm speed in the proximity of fish passages is likely a primary source of interference, but this interference is probably limited to a localized area where the propeller-induced wake remains intense with a large number of air bubbles being injected into the water column. Once the wake is dissipated into surface-drifting bubbles, there is little impact on fish migrating in deep water.

This study is unique in that it assesses reactions of actively migrating fish to survey vessels in a much shallower and confined underwater environment than a typical marine environment. The findings from this study may compliment those by Fernandes et al. (2000) obtained at sea where fish were distributed at depths beyond 40 m, which are much greater than the maximum depth of the lower Fraser River at Mission. While an interference range of 4 m might not be a concern when using the vessel to survey fish residing in mid- to deep waters at sea, the same interference range can have a pronounced effect on fish migrating in shallow waters or rising to the surface for their biological needs. This study provides a useful guide for mobile surveys, as the reported observations and findings might pertain to surveying fish with similar settings in a riverine or a shallow water environment.

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Long-term variation in isotopic baselines and implications for estimating consumer trophic niches

Christopher T. Solomon, Stephen R. Carpenter, James A. Rusak, and M. Jake Vander Zanden

Abstract: Carbon and nitrogen stable isotope ratios are increasingly used to study long-term food web change. Temporal variation at the base of the food web may impact the accuracy of trophic niche estimates, but data describing interannual baseline variation are limited. We quantified baseline variation over a 23-year period in a north-temperate lake and used a simulation model to examine how this variation might affect consumer trophic niche estimates. Interannual variation in C and N stable isotope ratios was significant for both benthic and pelagic primary consumer baselines. Long-term linear trends and shorter-term autoregressive patterns were apparent in the data. There were no correlations among benthic and pelagic C and N baselines. Simulations demonstrated that error in estimated fish trophic niches, but not bias, increased substantially when sampling of baselines was incomplete. Accurate trophic niche estimates depended more on accurate estimation of baseline time series than on accurate estimation of growth and turnover rates. These results highlight the importance of previous and continued efforts to constrain bias and error in long-term stable isotope food web studies.

Résumé : Les rapports d'isotopes stables de carbone et d'azote servent de plus en plus à évaluer les changements à long terme dans les réseaux alimentaires. Une variation temporelle à la base du réseau alimentaire peut avoir un impact sur la précision des estimations de niche trophique, mais il existe peu de données pour décrire la variation d'une année à l'autre de la ligne de base. Nous avons calculé la variation de la ligne de base sur une période de 23 ans dans un lac de la zone tempérée nord et nous utilisons un modèle de simulation pour examiner comment cette variation peut affecter les estimations des niches trophiques des consommateurs. La variation interannuelle des rapports d'isotopes stables de carbone et d'azote est significative pour les lignes de base des consommateurs primaires, tant benthiques que pélagiques. Il existe des tendances linéaires à long terme et des patrons d'autorégression à court terme dans les données. Il n'y a pas de corrélation entre les lignes de base de carbone et d'azote benthiques et pélagiques. Les simulations montrent que l'erreur, mais non la distorsion, dans les niches trophiques estimées des poissons augmente de façon substantielle lorsque l'échantillonnage des lignes de base est incomplet. Les estimations précises des niches trophiques dépendent plus d'une estimation précise de la série chronologique des lignes de base que de l'estimation précise des taux de croissance et de renouvellement de la biomasse. Ces résultats illustrent l'importance des efforts passés et actuels pour réduire l'erreur et la distorsion dans les études à long terme des réseaux alimentaires basées sur les isotopes stables.

[Traduit par la Rédaction]

Introduction

Understanding long-term change in the structure of food webs, and in the positions of consumers within food webs, is an important problem for ecological theory and application (Polis and Winemiller 1996; de Ruiter et al. 2005). Many important drivers of food web change act slowly, and thus, long-term studies are important tools for understanding their effects (Franklin 1989). Stable isotope analyses provide a powerful and increasingly used tool for describing food web change over long periods of time. Such studies have offered unique insights into the patterns, drivers, and consequences of variation in trophic structure, at temporal extents up to a century or more, in both marine (Wainright et al. 1993; Pruell et al. 2003; Becker and Beissinger 2006) and freshwater ecosystems (Vander Zanden et al. 2003; Gerdeaux and Perga 2006; Maguire and Grey 2006).

Stable isotope techniques are founded on three principles: consumers reflect the stable isotope ratios of their resources, basal food web resources are often isotopically distinct, and there is a relatively fixed change in isotope ratio with each trophic transfer between resource and consumer (Peterson and Fry 1987). By measuring the stable isotope ratios of a consumer and of basal resources (which are also referred to as food web "end members" or "baselines"), researchers can therefore quantify the consumer's trophic position and

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relative reliance on the different resources. In lakes, for instance, benthic and pelagic primary producers represent importantly different basal resources that also have distinct stable isotope ratios (France 1995; Hecky and Hesslein 1995). Measurements of the C and N stable isotope ratios of fishes reveal that many receive support from both benthic and pelagic food chains (Vander Zanden and Vadeboncoeur 2002).

Baseline stable isotope ratios vary over time. In lakes, planktonic and benthic primary consumers (zooplankton and zoobenthos) are commonly used as baselines (Vander Zanden and Rasmussen 1999; Post 2002). Many studies have demonstrated that physical, biogeochemical, and biological processes induce considerable within-year variability in zooplankton stable isotope ratios of C (Zohary et al. 1994; Gu et al. 1999; Leggett et al. 1999), N (Gu et al. 1994; Leggett et al. 2000), or both C and N (Yoshioka et al. 1994; Lehmann et al. 2004; Perga and Gerdeaux 2006). There is also evidence for within-year variation in zoobenthos stable isotope ratios (Grey et al. 2004; Hershey et al. 2006). Yet because most of these studies have focused on intraannual processes and have therefore spanned 2- to 3-year periods at most, data describing interannual variation in baseline stable isotope ratios are very limited. Available data indicate that C and N stable isotope ratios of zooplankton may vary interannually by several per mille (‰) or more (Zohary et al. 1994; Maguire and Grey 2006).

Researchers have recognized the need to consider how temporal baseline variation can affect interpretation of consumer stable isotope ratios. Accurate estimates of baselines are critical for distinguishing whether changes in consumer stable isotope ratios are due to ecological change or simply to variation in baseline isotope ratios (Cabana and Rasmussen 1996). For instance, a decrease in consumer $\delta^{15}N$ over time could indicate a shift by that consumer to feeding at a lower trophic position; or it could indicate a change in the inorganic N source utilized by primary producers, without any shift in the trophic position of the consumer. Unfortunately, assembling complete time series of baseline stable isotope data is often extremely challenging or impossible for long-term studies because of limited representation of aquatic primary consumers in many museum collections. Consequently, researchers have used a number of approaches to estimate baseline time series and to consider possible biases and errors. These include collecting multiple years of contemporary baseline isotope data (Pruell et al. 2003), using available historical museum samples of primary consumers either archived directly or dissected from the guts of preserved fish (Vander Zanden et al. 2003), estimating baselines from long-lived primary consumers that integrate temporal changes (Cabana and Rasmussen 1996), using supplementary data on environmental variables that suggest the presence or absence of change in baseline isotope ratios (Wainright et al. 1993; Gerdeaux and Perga 2006), and examining stable isotope ratios of organic matter in sediment cores (Chandra et al. 2005).

Such approaches have been instrumental in developing the unique insights that long-term food web studies can offer. Yet the magnitude and potential importance of the biases and errors that they seek to constrain remain unclear. To understand fully the implications of incomplete baseline data for long-term studies of consumer trophic niches, new data and analyses are needed. In this study, we present a 23-year time series of benthic and pelagic primary consumer baselines for a north-temperate lake. We fit statistical process models to these time series to derive general descriptions of the patterns of interannual variation. The empirical data provide the basis for a simulation model designed to assess how incomplete baseline data and inaccurate growth and tissue turnover data affect bias and precision of consumer trophic niche estimates in long-term food web studies.

Materials and methods

Collection and statistical modeling of empirical data

We measured C and N stable isotope ratios ($\delta^{13}C$ and $\delta^{15}N$; henceforth, collectively δX) in archived samples of benthic and pelagic primary consumers collected from Sparkling Lake, Wisconsin, between 1981 and 2003 by the North Temperate Lakes LTER project (see Magnuson et al. 2006). Primary consumers provide good isotopic baselines because they integrate over some of the variability in primary producer stable isotope ratios (Vander Zanden and Rasmussen 2001; Post 2002).

Benthic macroinvertebrates collected with Dendy colonization samplers during August of each year were preserved in 70% ethanol (lter.limnology.wisc.edu/dendy05.shtml). Individual gammarid amphipods and heptageniid mayflies of the genus *Stenonema* were removed from these samples, rinsed with distilled water, dried at 60 °C for 1 day, and analyzed whole on an isotope ratio mass spectrometer at the University of California Davis Stable Isotope Facility (Davis, California). We selected these two taxa for analysis so that our benthic baseline would reflect changes in the isotope ratios of both detrital and algal resources in benthic habitats. Individuals were analyzed singly when possible or in small groups when necessary to achieve sufficient mass. We ran one to three samples of each of these taxa of zoobenthos in each year (except 1989, when no samples were available).

Depth-integrated zooplankton samples were collected with a Wisconsin net throughout the year, predominantly during the ice-free season (80 μm mesh, n usually between 4 and 8 depending on the year), and preserved in 3%–5% formalin (prior to 2001) or 80% ethanol (2001–2003) (lter.limnology.wisc.edu/bio_field_protocol05.shtml). We representatively subsampled these samples for isotopic analysis after filtration through a 250 μm mesh to ensure that only larger zooplankton (primarily *Daphnia* and large copepod species) were analyzed isotopically.

To account for the effects of sample preservation, we adjusted observed zooplankton $\delta^{13}C$ and $\delta^{15}N$ by +1.1‰ and +0.8‰ when samples were preserved in formalin and by +0‰ and +0.8‰ when samples were preserved in ethanol (Feuchtmayr and Grey 2003). We did not adjust observed $\delta^{13}C$ or $\delta^{15}N$ of zoobenthos (Sarakinos et al. 2002).

We described patterns of variation in time series of baseline δX by comparing fits of the data to four plausible process models consistent with current thinking about isotope behavior in food webs (Table 1). The simplest model describes each year's δX as a random draw from a normal distribution; this represents the pattern of baseline variation

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Table 1. Comparison of fits for each of four model types fit to each of four time series of baseline stable isotope data.

	Zoobenthos $\delta^{13}C$	Zoobenthos $\delta^{15}N$	Zooplankton $\delta^{13}C$	Zooplankton $\delta^{15}N$
Model 1: $Y_t = B_0 + \varepsilon_t$	86.4	31.6	94.3	73.5
Model 2: $Y_t = B_0 + B_1 \times t + \varepsilon_t$	79.6	31.2	96.1	59.3
Model 3: $Y_t = \phi \times Y_{t-1} + \varepsilon_t$	90.9	34.6	104.7	63.4
Model 4: $Y_t = B_0 + B_1 \times t + \phi \times Y_{t-1} + \varepsilon_t$	81.5	31.0	97.3	58.8

Note: Table values give the Akaike information criterion for each fit; lower values indicate better fits. Y_t is the mean stable isotope ratio of C or N in zoobenthos or zooplankton in year t, ε_t is the error at time t, with $\varepsilon \sim N(0,\sigma^2)$, B_0 and B_1 are parameters for the intercept and slope of a linear regression, and ϕ is an autoregressive memory parameter describing the strength of the connection between the stable isotope ratio in year t and year $t-1$.

that is implicitly assumed by many retrospective stable isotope studies. Two models of intermediate complexity describe δX as changing either linearly or autoregressively over time. Finally, the fourth model includes both a linear trend and an autoregressive memory term. We fit each model to each baseline time series by maximum likelihood, compared models using the Akaike information criterion, and generated confidence intervals for parameter estimates using nonparametric bootstrapping. For most of our analyses, we considered only zooplankton samples collected during the ice-free season each year (usually $n = 4$–6), as this probably represents the most relevant pelagic baseline estimate for fish growth (Perga and Gerdeaux 2005). However, we also considered whether model fits were qualitatively different if we included all zooplankton samples or only summertime (May through August) samples.

Simulation model

When isotopic baseline variation occurs at a time scale similar to that of the ecological process of interest, researchers must consider both the time series of baseline δX and the consumer's rates of tissue growth and turnover to interpret consumer stable isotope ratios accurately (Harvey et al. 2002; Carpenter et al. 2005; Matthews and Mazumder 2005). This is because the δX of a given fish in a given year depends both on the baseline δX during each year of its life and on the proportion of its current biomass that comes from each year's growth. These "mass proportions", in turn, are determined by tissue growth and turnover rates. In long-term studies, researchers typically estimate (explicitly or implicitly) both of these inputs: they interpolate a baseline time series from available data and approximate consumer growth and turnover rates with reasonable guesses. In addition, they estimate the isotopic fractionation associated with trophic transfers from prey to predator. We developed a simulation model to explore how the accuracy of these inputs affects the accuracy and precision of a researcher's inferences about the consumer's trophic niche. The model is described briefly here; additional details and a flow chart are given in Appendix A.

The first phase of the model tracks a population of fish through time, calculating the δX of the average individual of each age in each year. The second phase mimics the implementation of a research project interested in long-term change in consumer trophic niches (specifically τ, the trophic position, and p, the percent reliance on benthic resources). A "researcher" measures the δX of fish from each year and calculates their p and τ using estimates of their mass proportions, the trophic fractionation of isotope ratios, and the baseline δX time series. As in a real study, the researcher's estimates of these three factors may be imperfect.

We quantified the biases (systematic departures from the true value) and errors (magnitude of scatter around the true value) in $\hat{p}$ and $\hat{\tau}$ across multiple scenarios in which we varied the quality of the researcher's information about these three factors. For the estimated baseline time series, we varied the proportion of years in which the researcher measured baseline δX between one and all of the 23 years of the time series. We also varied whether the sampled years were spaced randomly or evenly and whether the researcher calculated values for unsampled years by linear interpolation or as the mean of sampled years. For the estimated mass proportions, we considered four levels of accuracy: (*i*) the true mass proportions using the nominal growth and turnover rates, (*ii*) the worst possible estimate of mass proportions when growth and turnover rate estimates were constrained within ±20% of the nominal rates (determined by maximizing sums of squares), (*iii*) equal mass proportions (that is, assuming that each year of the consumer's life makes an equal contribution to its mass), and (*iv*) the worst possible mass proportions when growth and turnover rate estimates were unconstrained (i.e., estimated within 50%–200% and 10%–1000% of the nominal rates, respectively). For the estimated trophic fractionation of C and N isotope ratios, we ran simulations in which the true baseline time series and true trophic fractionation remained fixed, but in each model iteration, the researcher randomly drew estimates of the C and N trophic fractionation from a normal distribution. These distributions were centered on the true trophic fractionations, with standard deviations (1.04‰ for C and 0.41‰ for N) described by field estimates of the variance in trophic fractionation (Vander Zanden and Rasmussen 2001).

We focused primarily on the effects of baseline and mass proportions estimates because the effects of uncertainty in trophic fractionation have been addressed elsewhere (Vander Zanden and Rasmussen 2001; Post 2002; Matthews and Mazumder 2005). For each scenario, we ran 100 realizations, generating new baseline δX time series each time. In each realization, we calculated the absolute mean bias ("bias") and the standard deviation ("error") of $\hat{p}$ and $\hat{\tau}$ across years for fish of each age class. We then calculated the mean bias and error across the 100 realizations so that we present estimates of the bias and error in $\hat{p}$ and $\hat{\tau}$ across the 23-year time series that do not depend on a particular realization of the baseline-generating process but incorporate the overall effect of that process.

Results

Empirical data

All four of the Sparkling Lake baseline time series

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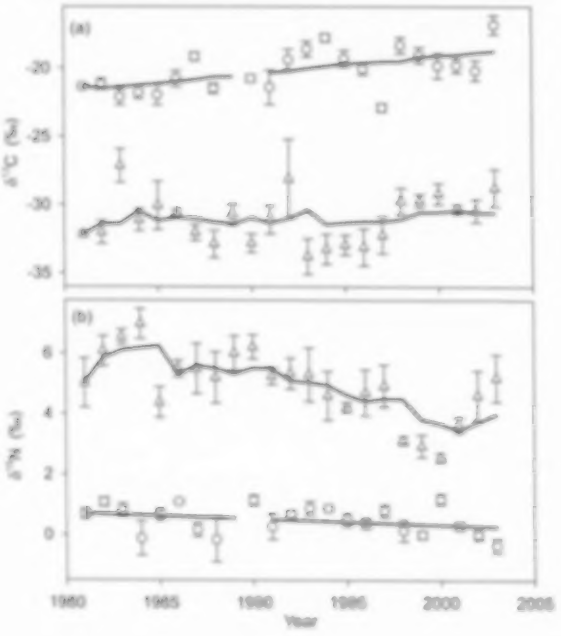
Fig. 1. Mean annual stable isotope ratios of (*a*) C and (*b*) N of food web baselines (circles, zoobenthos; triangles, zooplankton) in Sparkling Lake, Wisconsin. Error bars are ± 1 SE. Lines show predictions from the fitted model (model 4), which incorporates a linear trend and temporal autocorrelation.



(Fig. 1) exhibited significant interannual differences in stable isotope ratios (ANOVA: df = 22, 143 for zooplankton $\delta^{13}C$ and $\delta^{15}N$, df = 21, 71 for zoobenthos $\delta^{13}C$, and df = 21, 70 for zoobenthos $\delta^{15}N$, $p < 0.0003$ in all cases). This was true even though seasonal variation resulted in large intraannual variances in zooplankton δX. Pooled intraannual variances were 5.95‰ and 1.15‰ for zooplankton $\delta^{13}C$ and $\delta^{15}N$, respectively, and 1.15‰ and 0.22‰ for zoobenthos $\delta^{13}C$ and $\delta^{15}N$, respectively. Changes in baseline stable isotope ratios were uncorrelated within habitats (benthic versus pelagic) and within elements (C versus N) (Table 2). Both $\delta^{15}N$ time series trended downward over time; in contrast, there was little directional change in zooplankton $\delta^{13}C$ and an upward trend in zoobenthos $\delta^{13}C$ (Fig. 1).

Zooplankton and zoobenthos baselines were clearly different in both C and N stable isotope ratios. On average, mean annual zooplankton $\delta^{13}C$ was 9.94‰ lower than mean annual zoobenthos $\delta^{13}C$ (paired *t* test: $p < 0.0001$), and mean annual zooplankton $\delta^{15}N$ was 5.20‰ higher than mean annual zoobenthos $\delta^{15}N$ (paired *t* test: $p < 0.0001$).

Only one time series (zooplankton $\delta^{13}C$) was best described by model 1, the completely random model. Fits to all three of the remaining time series improved substantially with the inclusion of a linear trend in the model, and fits for the two $\delta^{15}N$ time series were improved further by inclusion of an autoregressive memory term (Table 1). There was little qualitative difference in these patterns when we fit models to year-round or summer-only zooplankton data instead of to data from the ice-free season or when we fit models to data without correcting for effects of preservation in ethanol and formalin (results not shown).

Table 2. Correlations (Pearson's *r*) among observed time series of baseline stable isotope ratios and *p* values for test of null hypothesis that correlation is zero.

Comparison	*r*	*p*
Within elements		
Zoobenthos $\delta^{13}C$ and zooplankton $\delta^{13}C$	0.03	0.9
Zoobenthos $\delta^{15}N$ and zooplankton $\delta^{15}N$	0.08	0.7
Within habitats		
Zoobenthos $\delta^{13}C$ and $\delta^{15}N$	–0.25	0.3
Zooplankton $\delta^{13}C$ and $\delta^{15}N$	–0.12	0.6

For the purposes of the simulation modeling, we chose to describe all four time series using the most complex model (model 4). All of the simpler models are nested within model 4; thus, by setting some parameters equal to or near zero, model 4 can yield predictions similar to those made by model 2 for zoobenthos $\delta^{13}C$ and by model 1 for zooplankton $\delta^{13}C$. Maximum likelihood parameter estimates, with bootstrapped 95% confidence intervals, are presented for all four time series fit to model 4 (Table 3).

Simulation model

Bias and error in trophic niche estimates declined as the proportion of sampled baseline years increased (Fig. 2). This result held regardless of whether the sampled years were spaced randomly or evenly across the time series and regardless of whether values for unsampled years were inferred by linear interpolation or as the mean of sampled years. For simplicity, we present data only from simulations in which sampled years were evenly spaced and unsampled years were inferred by averaging. Fish age had little effect on bias in $\hat{p}$ and $\hat{\tau}$, but errors were smaller for older fish because the importance of incorrect baseline estimates in any given year was muted by the larger number of years contributing to the biomass of older fish. Bias was relatively low as long as >50% of the baseline years were sampled (<0.02 for $\hat{p}$ and <0.07 for $\hat{\tau}$ for age-5 fish) but increased rapidly as the proportion of sampled years declined below 30%. In contrast, errors in $\hat{p}$ and $\hat{\tau}$ increased rapidly in response to reductions in baseline sampling to maxima of 0.07 and 0.2, respectively (Fig. 2).

The manifestation of such errors in a particular study could lead to an erroneous inference of variability or long-term change in trophic niche. For example, output is shown from a typical realization of the model scenario in which mass origins were known perfectly and 22% of the years in the baseline time series were sampled (Fig. 3). Although the mean of $\hat{p}$ and $\hat{\tau}$ across the 23-year time series was close to true *p* and τ, the value of $\hat{p}$ and $\hat{\tau}$ in any given year often differed from the true value. For age-1 fish, estimated trophic position was off by more than half a trophic level in seven separate years and by a full trophic level in two years (Fig. 3*b*); $\hat{p}$ was often off by >0.10 (Fig. 3*a*). For age-5 fish, error in $\hat{p}$ and $\hat{\tau}$ in a given year was much less than for age-1 fish as a result of isotopic integration across years. Interestingly, $\hat{p}$ showed a significant increase over time for age-5 fish in this realization even though true *p* remained

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Table 3. Maximum likelihood estimates and bootstrapped 95% confidence intervals (in parentheses) for parameters of the statistical model (model 4) used to describe interannual variation in $\delta^{13}C$ and $\delta^{15}N$ of zooplankton and zoobenthos.

	B_0	B_1	ϕ	σ^2
Zoobenthos $\delta^{13}C$	−20.89 (−22.98, −13.99)	0.13 (0.04, 0.21)	0.04 (0.00, 0.34)	1.43 (0.47, 2.03)
Zoobenthos $\delta^{15}N$	0.71 (0.26, 1.12)	−0.02 (−0.05, 0.01)	0.00 (0.00, 0.33)	0.16 (0.07, 0.21)
Zooplankton $\delta^{13}C$	−25.55 (−32.90, −21.64)	0.02 (−0.10, 0.14)	0.18 (0.00, 0.30)	2.84 (1.05, 4.18)
Zooplankton $\delta^{15}N$	4.33 (4.45, 7.18)	−0.08 (−0.18, −0.07)	0.33 (0.00, 0.32)	0.53 (0.21, 0.88)

Note: Parameters are intercept (B_0) and slope (B_1) for a linear trend, a memory parameter (ϕ) describing temporal autocorrelation, and the variance (σ^2) of the normally distributed errors.

Fig. 2. Bias (mean absolute bias, circles) and error (SD, triangles) in estimates of trophic niche resulting from incomplete sampling of baseline time series. (*a*) Bias and error in $\bar{p}$, the estimated proportional reliance of the consumer on benthic resources; (*b*) bias and error in $\bar{\tau}$, the estimated trophic position of the consumer. Each point represents the mean across 100 model realizations of the summary statistic (bias or SD) calculated across a 23-year time series for age-5 fish.

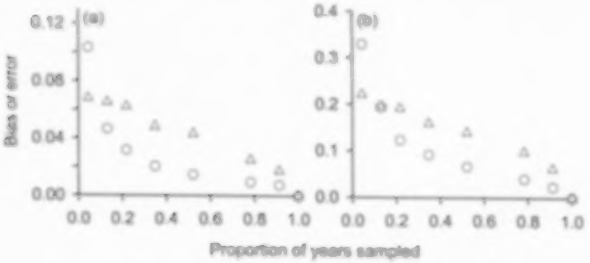
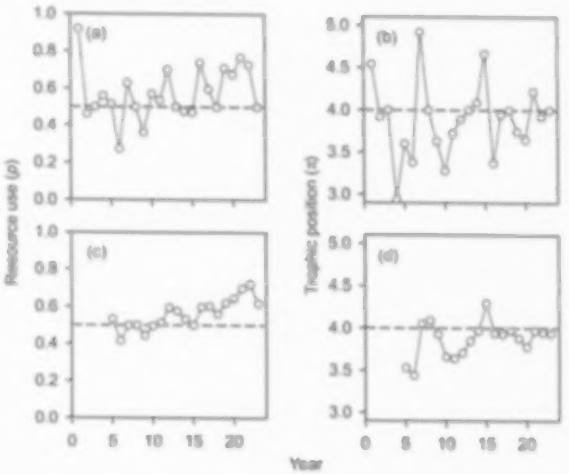


Fig. 3. Time series of estimated proportional reliance on benthic resource ($\bar{p}$) and estimated trophic position ($\bar{\tau}$) for (*a* and *b*) age-1 fish and (*c* and *d*) age-5 fish in one realization of the simulation model. True p and τ, indicated by the broken line, were held constant at 0.5 and 4.0, respectively. Data are from a model scenario in which estimated growth and turnover rates equal the nominal rates but baseline time series are sampled every fifth year (22% of years).



constant, as a result of the vagaries of the estimated baseline time series (Fig. 3*c*) ($p = 0.002$).

Inaccurate estimates of fish growth and turnover rates generally inflated bias and error in trophic niche estimates; however, this inflation was small as long as growth and turnover rates were reasonably bounded (Fig. 4). Even the worst possible growth and turnover estimates within ±20% of the true rates produced little difference in the estimated mass proportions of the fish (Fig. 4*a*) and therefore had little effect on bias or error in $\bar{p}$ and $\bar{\tau}$ (Figs. 4*b* and 4*c*). On the other hand, when growth and turnover rate estimates were grossly inaccurate (amounting to a misspecification of the form of the growth model, not just of the parameters), inflation in bias, and particularly error, was more substantial (Fig. 4, scenarios *iii* and *iv*). Inflation of error and bias were largest when sampling of the baseline time series was complete and decreased as the quality of the researcher's estimate of the baseline time series decreased (that is, as the proportion of sampled baseline years decreased) (Fig. 4).

Uncertainty about the true trophic fractionation increased the mean bias, but not the error, in trophic niche estimates. Compared with scenarios in which the true trophic fractionation was known, the mean biases in $\bar{p}$ and $\bar{\tau}$ were, respectively, 0.09–0.18 and 0.04–0.27 greater when trophic fractionation was estimated. This bias inflation was not sensitive to the completeness with which the baseline time series was sampled.

Discussion

Empirical data

Stable isotope ratios of food web baselines vary at multiple time scales. To characterize annual values, we analyzed multiple samples through time for short-lived pelagic primary consumers and replicate samples of longer-lived benthic primary consumers. The point estimates that we report for annual values therefore integrate over much of the isotopic variability at time scales shorter than a year. Despite the uncertainty in these point estimates (intraannual errors), we observed significant interannual variation in $\delta^{13}C$ and $\delta^{15}N$ of both benthic and pelagic baselines in Sparkling Lake.

Patterns of baseline variation not only provide clues to the mechanisms that drive such variation, but also determine the sampling frequency necessary to adequately characterize baseline time series. For three of the four baseline time series in Sparkling Lake, interannual variation was better described by statistical models that included long-term linear trends and (or) temporal autocorrelation than by purely random models. This indicates that the observed variation may have resulted from slow, directional shifts in key driver

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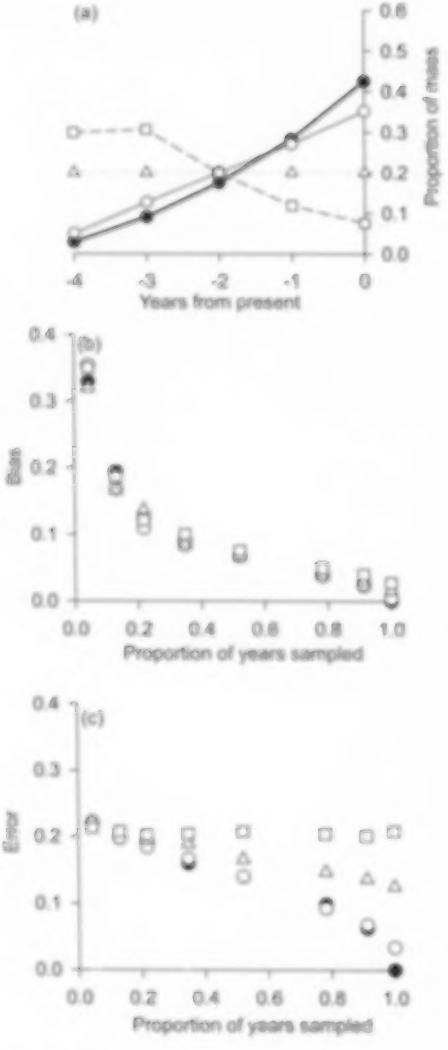
Fig. 4. Effect of growth and turnover rate estimates on bias and error in consumer trophic niche estimates. (*a*) Proportion of the current mass of an age-5 fish that is derived from each year of its life in four scenarios with different growth and turnover rates. Scenarios are (*i*) nominal growth and turnover rates (solid circles), (*ii*) the worst possible estimate of mass proportions when growth and turnover rate estimates are constrained within ±20% of the nominal rates (open circles), (*iii*) growth and turnover are not explicitly estimated, but mass proportions are assumed to be uniform across the consumer's life (triangles), and (*iv*) the worst possible mass origins when growth and turnover rate estimates were unconstrained (within 50%–200% and 10%–1000% of the nominal rates, respectively) (squares). (*b*) and (*c*) Bias and error in $\bar{\tau}$, the estimated trophic position of the consumer, plotted against the proportion of baseline years sampled for each of the growth scenarios shown in Fig. 4*a*.



processes rather than from stochastic environmental noise around a stationary mean. Although our results provide insight on the statistical form of the processes that drive long-term baseline change in Sparkling Lake, the identity of these drivers remains unclear. Two potential mechanisms can be excluded. First, the "Seuss effect", a long-term decline of about 0.016‰·year^{-1} in the $\delta^{13}C$ of atmospheric CO_2 owing to fossil fuel combustion, could be dampening, but not driving, the slight increase in $\delta^{13}C$ that we observed for both zooplankton and zoobenthos (Kroopnick 1985; Quay et al. 2003). Second, although the structure of the Sparkling Lake zooplankton community changed considerably in the early 1980s (to dominance by cyclopoids as a result of an invasive planktivorous fish; Beisner et al. 2003) and although shifts in zooplankton community structure can alter the stable isotope ratios of zooplankton baselines (Matthews and Mazumder 2003), this change does not seem to be detectable in the zooplankton isotopic time series. A variety of other physical, biogeochemical, and biological processes could be driving baseline variation. For zooplankton, a number of these processes have been identified in studies spanning one to a few years (see citations in the Introduction). In Sparkling Lake, the lack of correlation among the time series suggests that either the identity or the effects of these key processes vary among the four habitat-by-element combinations that we considered. Complete analyses of potential drivers, using multiple long-term data sets such as this one, will be necessary to fully understand the causes of interannual variation in baseline stable isotope ratios. Such information could be extremely useful for predicting changes in baselines even when historical samples of baseline organisms are unavailable.

Because long-term baseline data sets are scarce, it is difficult to assess how well the patterns that we observed might represent baseline variation in other systems. As a first cut at comparing interannual baseline variation among systems, we fit the four statistical process models that we considered in this paper to a continuous 19-year time series of zooplankton stable isotope data from Lough Erne (Maguire and Grey 2006, data digitized from their fig. 1). These data come from a single June sample in each year and include a much broader size range of zooplankton than our samples; thus, they may not be directly comparable with our zooplankton data, although the range of variation in $\delta^{13}C$ and $\delta^{15}N$ was similar in the two data sets. As in Sparkling Lake, the random model was the best fit to the Lough Erne zooplankton $\delta^{13}C$ time series, with both the linear and linear-plus-autoregressive models as plausible alternatives. The linear model was clearly the best fit for the Lough Erne zooplankton $\delta^{15}N$ time series; in Sparkling Lake, this model explained variation in zooplankton $\delta^{15}N$ almost as well as the selected linear-plus-autoregressive model. Although this analysis has significant limitations, it does suggest that similar statistical processes may describe interannual baseline variation in different lakes. Identifying key processes that drive variation will enable better comparisons among lakes. For instance, if winter under-ice respiration affects algal $\delta^{13}C$ by setting the $\delta^{13}C$ of the dissolved inorganic C pool in the springtime, then lakes with similar dissolved inorganic C pool sizes, morphometry, and ice duration may show some coherence in their interannual baseline $\delta^{13}C$.

Simulation model

Although inaccurate estimates of consumer growth and

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turnover rates did result in inaccurate trophic niche estimates, this effect was strong only when rate estimates were highly imprecise; when rate estimates were reasonably bounded, errors in mass proportions were small and had little effect on trophic niche estimates. Reasonably bounded estimates of growth and turnover rates are usually possible, even in the absence of data specific to the study system, because these processes have been studied extensively. Well-tested models of fish growth and even species-specific parameter values for these models are widely available (Quinn and Deriso 1999; Froese and Pauly 2006). Data describing tissue turnover rates are also substantial and growing rapidly (e.g., Hesslein et al. 1993; MacAvoy et al. 2001; McIntyre and Flecker 2006). Thus, inaccurate estimates of consumer growth and turnover rates will likely be negligible sources of bias and error in most long-term studies.

Bias and error resulting from incomplete baseline data may be more important concerns for long-term stable isotope studies than those resulting from inaccurate growth and turnover estimates. In many cases, researchers are interested in understanding changes in consumer trophic niches over long periods during which baseline samples are scarce. For instance, in a previous study in which we sought to understand food web change and restoration potential in Lake Tahoe, we had baseline data available for only two of five time periods spanning >100 years (Vander Zanden et al. 2003). Results from the present study suggest that incomplete baseline data may affect consumer trophic niche estimates, particularly by introducing error. On the positive side, biases in trophic niche estimates resulting from incomplete baseline data will be minimal in many cases. Nonetheless, our results underscore the importance of the measures that researchers have taken to assess and limit biases and errors in consumer trophic niche estimates from long-term stable isotope studies.

Quantitative estimates of the bias and error attributable to incomplete baseline data depend on the extent and pattern of variability in the baseline time series. The simulation results reported here are based on baseline time series similar to those that we observed in Sparkling Lake. To make our results as general as possible, we did not rely strictly on the observed data but instead generated multiple realizations of baseline time series based on the statistical processes that best described the data. Nonetheless, even these generalized statistical process models are parameterized based only on one lake. Our quantitative estimates of bias and error should therefore be interpreted with caution. Qualitatively, however, the simulation results reported here may be quite general. For instance, we observed that biases increased slowly in response to decreases in available baseline data until sampling became quite sparse and that errors increased somewhat more quickly. These patterns occur because small gaps in baseline data do not significantly decrease the overall accuracy of the estimated baseline time series but can introduce localized errors in trophic niche estimates for consumers in the years when baseline data are not available. Similar patterns would be obtained even in systems with patterns of baseline variation different from those observed in Sparkling Lake.

Even when baseline sampling is very sparse, bias from inaccurate baseline estimates is less than what may result strictly from inaccurate trophic fractionation estimates. For example, in our simulations, realistic levels of uncertainty in trophic fractionation resulted in increases of 0.09–0.18 in mean bias in $\bar{p}$ relative to scenarios in which trophic fractionation was known. In comparison, the mean bias in $\bar{p}$ owing to inaccurate estimates of the baseline time series was <0.11 even when baseline data were available from only 4% of the years of the time series. Other recent studies have observed similar biases in $\bar{p}$ and $\bar{\tau}$ as a result of uncertainty in trophic fractionation (Vander Zanden and Rasmussen 2001; Post 2002).

Researchers who use long-term stable isotope techniques have recognized the need to interpret consumer trophic niche estimates with caution when baseline data are incomplete (Wainright et al. 1993; Vander Zanden et al. 2003; Matthews and Mazumder 2005). However, the limited availability of long, complete baseline time series has precluded quantitative assessment of potential biases and errors. Our empirical data and simulation analysis indicate that long-term stable isotope studies will need to continue to consider issues including baseline data availability, growth and turnover models, and trophic fractionation uncertainty. In addition, there is a clear need for more data describing long-term variation in isotopic baselines and for improved capabilities for understanding and predicting such variation. Such advances will continue to expand the ability of long-term stable isotope studies to reveal insights into food web pattern and process in aquatic systems.

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Appendix A. Detailed description of simulation model

We used a simulation model (Fig. A1) to explore how estimates of consumer trophic niches are affected by the accuracy of (*i*) estimated time series of stable isotope ratios in food web baselines and (*ii*) estimated tissue growth and turnover rates of the consumer.

In the first phase of the model, three pieces of information are used to calculate fish δX for each year and age class. First, the fish's trophic niche determines the proportion of its biomass that comes from benthic versus pelagic resources and the number of trophic transfers (and associated isotopic fractionations) that occur between the resources and the fish. The particular values chosen for p and τ are of little consequence because the objective of the model is to determine how accurately the researcher can estimate p and τ (whatever they may be) given imperfect estimates of growth, turnover, and baseline δX. We set $p = 0.5$ and $\tau = 4.0$. Isotopic fractionation occurs with each trophic transfer between end members and fish. Empirical estimates of trophic fractionation vary (Vander Zanden and Rasmussen 2001; McCutchan et al. 2003; Vanderklift and Ponsard 2003), and the effects of this uncertainty on consumer trophic niche estimates have been considered by several authors (Vander Zanden and Rasmussen 2001; Post 2002; Matthews and Mazumder 2005). As a point of comparison, we also investigated these effects in one set of simulations (see description below), but for most simulations, we set trophic fractionation constant at 0.8‰ for C and 3.4‰ for N (Vander Zanden and Rasmussen 2001).

Second, varying baselines mean that the isotope ratios of new tissue depend on the year in which tissue is added. Rates of tissue growth and turnover control the contribution of each year's growth to the total biomass of the fish; we call this the fish's "mass proportions". Again, the particular values for growth and turnover parameters are relatively unimportant because we focus on how the researcher's estimates of these parameters affect the accuracy of $\bar{p}$ and $\bar{\tau}$. We defined fish growth according to a von Bertalanffy age–length relationship and an allometric length–weight relationship using parameter values for yellow perch (*Perca flavescens*), a common north-temperate species. Parameter values were taken from Carlander (1950) and Froese and Pauly (2006) based on original work by Smith (1939) and Hasler (1945). Fish replaced 30.6% of their tissue every year via metabolic turnover based on a daily turnover rate of 0.1% (Hesslein et al. 1993).

Third, given the trophic niche and rates of growth and turnover, fish stable isotope ratios are determined by the baseline δX time series. We assumed that the observed Sparkling Lake baseline data were just one realization of a stochastic process that generates time series of baseline δX. To make our conclusions about the effects of incomplete baseline data as generalizable as possible, we ran multiple iterations of each model scenario and generated a new realization of the baseline time series in each iteration. Each realization was generated using the statistical model structure that best fit the observed Sparkling Lake data, but each time, the parameter values for the model were selected randomly from the set of bootstrapped parameter estimates (see main text: Methods, Collection and statistical modeling of empirical data). Inter- and intra-annual errors (drawn from empirically estimated distributions) were also added to each baseline realization. Thus, each iteration of the simulation model uses a plausible realization of baseline δX time series that incorporates our uncertainty about both the underlying generating process and the observed data in a given year.

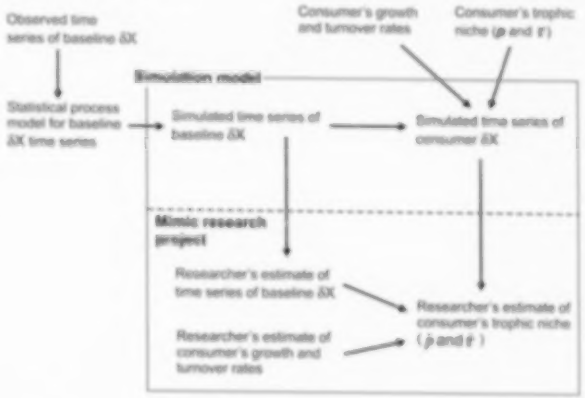
Observed time series of baseline δX
Consumer's growth and turnover rates
Consumer's trophic niche (p and τ)
Simulation model
Statistical process model for baseline δX time series
Simulated time series of baseline δX
Simulated time series of consumer δX
Mimic research project
Researcher's estimate of time series of baseline δX
Researcher's estimate of consumer's trophic niche (p and τ)
Researcher's estimate of consumer's growth and turnover rates

Fig. A1. Flow chart describing steps in the simulation model. The goal of the model is to assess bias and error in estimates of consumer trophic niches (τ, trophic position, and p, the proportional reliance of the consumer on benthic resources) that result from inaccurate estimates of either the true baseline time series or the consumer's growth and turnover rates. The first phase of the model (above the broken line) uses three types of inputs to simulate time series of baseline and consumer stable isotope ratios in C and N (δX). The second phase (below the broken line) mimics the implementation of a research project in which a researcher samples from the baseline and consumer δX time series and calculates estimates of p and τ ($\bar{p}$ and $\bar{\tau}$).

Up to this point, the model is concerned with calculating the "true" δX for fish of each age in each year, given the "true" baseline time series, growth and turnover rates, and trophic niche of the fish. The second phase of the simulation model mimics the procedure that a researcher would use to calculate $\bar{p}$ and $\bar{\tau}$ in a long-term study (Fig. A1). The researcher samples fish δX without ,error (for simplicity) but may have imperfect estimates of the baseline time series and the mass proportions of the fish. Given the age and stable isotope signature of a fish, the researcher uses the estimated mass proportions and baseline time series to estimate the trophic niche of the fish. This estimate is calculated by using an iterative procedure to find values of $\bar{p}$ and $\bar{\tau}$ that

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minimize the squared distance (in $\delta^{13}C$, $\delta^{15}N$ space) between observed and predicted fish δX (Post 2002).

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Maternal transfer of carbon and nitrogen to progeny of sea-run and resident brook trout (*Salvelinus fontinalis*)

Timothy D. Jardine, Eric Chernoff, and R. Allen Curry

Abstract: Early-life history characteristics of sea-run and resident salmonid fishes that co-exist in sympatry are poorly understood. To assess differences in characteristics of offspring of these two strategies following their emergence to the stream, stable isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) were used to identify the progeny of resident and sea-run female brook trout (*Salvelinus fontinalis*) in a tributary of the Miramichi River, New Brunswick, Canada. Upon emergence, sea-run progeny captured in emergence traps were longer, heavier, and had higher carbon to nitrogen ratios (C/N, an indicator of lipid content) than resident progeny, but did not have a higher body condition. After some feeding and growth in the stream, sea-run progeny (still identifiable by their $\delta^{13}C$ and $\delta^{15}N$) captured in dip-nets became longer, heavier, had higher C/N, and had higher condition than resident progeny. The proportion of fish caught in dip nets at sites where the two forms co-existed was dominated by offspring of sea-run females (sea-run = 56%, resident = 19%, unknown = 25%). These analyses suggest that offspring of sea-run, female trout benefit from maternally derived energy reserves gained at sea and thus have potential adaptive advantages over offspring of residents.

Résumé : Les caractéristiques des premières étapes du cycle des poissons salmonidés anadromes et résidants qui vivent en sympatrie restent mal comprises. Pour évaluer les caractéristiques différentes des rejetons issus de ces deux stratégies après leur émergence dans le cours d'eau, nous avons utilisé les rapports d'isotopes stables ($\delta^{13}C$ et $\delta^{15}N$) pour distinguer les rejetons des femelles résidantes et anadromes de l'omble de fontaine (*Salvelinus fontinalis*) dans un tributaire de la Miramichi, Nouveau-Brunswick, Canada. À l'émergence, les rejetons anadromes capturés dans les pièges d'émergence sont plus longs et plus lourds et possèdent un rapport carbone sur azote (C/N, un indicateur du contenu lipidique) plus élevé que les rejetons résidants, mais ils n'ont pas une meilleure condition corporelle. Après s'être alimentés et avoir fait de la croissance dans le cours d'eau, les rejetons anadromes (encore identifiables par leurs $\delta^{13}C$ et $\delta^{15}N$) capturés dans des épuisettes sont devenus plus longs et plus lourds et possèdent un rapport C/N et une condition plus élevés que les rejetons résidants. Les proportions des poissons capturés par épuisette dans les sites dans lesquels les deux formes cohabitent sont dominées par les rejetons des femelles anadromes (anadromes = 56 %, résidants = 19 %, inconnus = 25 %). Ces analyses laissent croire que les rejetons des ombles femelles anadromes tirent bénéfice des réserves d'énergie d'origine maternelle accumulées en mer et qu'ils possèdent ainsi un avantage adaptatif potentiel par rapport aux rejetons des résidants.

[Traduit par la Rédaction]

Introduction

In coastal streams of temperate regions around the world, many populations of fishes in the family Salmonidae exist as two forms in sympatry, with resident fish completing their life cycle in the natal stream and anadromous or sea-run fish migrating to saline waters before returning to the stream to spawn (Jonsson and Jonsson 2001). Migration to more productive saline waters often yields larger body sizes in sea-run fish (Tallman et al. 1996). Because of predictable relationships between female body size and fecundity (Morita and Takashima 1998), larger anadromous females have an assumed adaptive advantage of increased fecundity over residents (Tallman et al. 1996). Sea-run fish, however, are believed to have higher mortality rates associated with the challenges of migration from headwater streams to estuaries (e.g., areas with higher rates of predation by birds and mammals; Dieperink et al. 2002) and intense human fishing pressure where anglers can disproportionately target sea-run fish because of their larger body size (Roche 1992).

It is generally unknown whether potential adaptive advantages exist among different forms in the juvenile stage, particularly in the period between emergence and the end of their first year (Perry et al. 2005). Given the high rates of mortality in salmonids during the first year of life (Hutchings and Jones 1998), any competitive or survival advantages at this time could lead to selection favouring one form over the other (Hutchings 1991). Body size, lipid reserves, and condition are three relevant measures of fitness in juvenile fishes because of their importance for over-winter survival, particularly in northern latitudes (Finstad et al. 2004). Despite the importance of understanding differences between forms in early life, their identification

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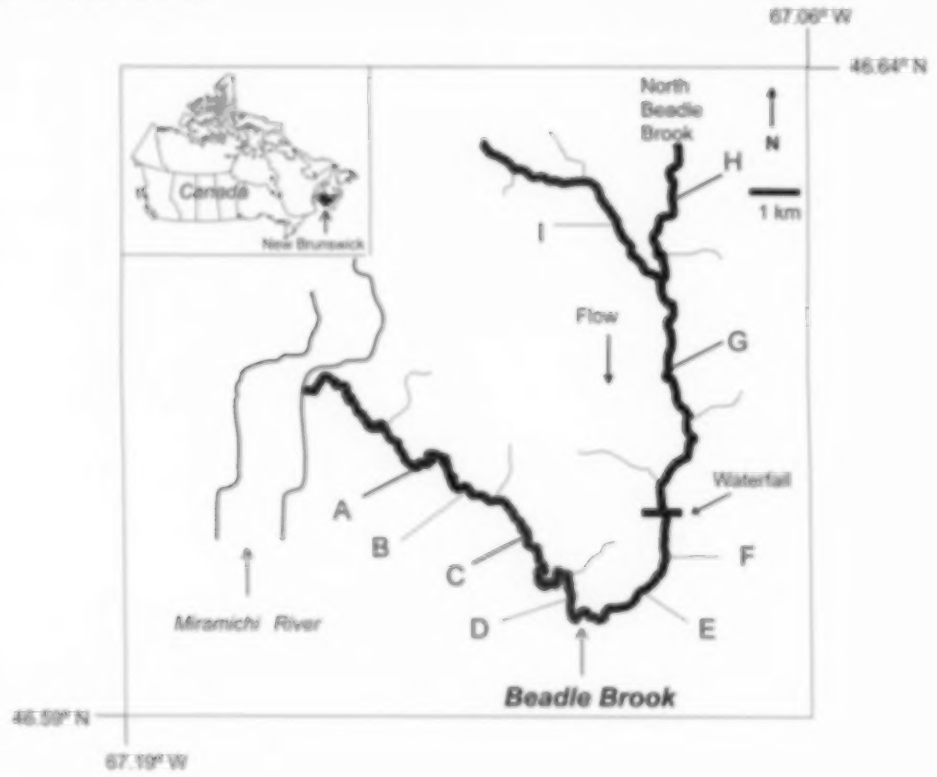
Fig. 1. Location of the study area: Beadle Brook, New Brunswick, Canada. Sites A–F are below a waterfall that is impassable to migratory fishes. Sites G–I are above the waterfall.



and separation has presented a major challenge using conventional techniques (Rieman et al. 1994; Thériault and Dodson 2003).

Stable isotope analysis (SIA) has emerged as a tool to identify migratory vs. resident individuals in fish populations (Doucett et al. 1999*a*; McCarthy and Waldron 2000; Jardine et al. 2005*a*). SIA more recently has been used to trace the movement of marine organic matter into eggs and ultimately to emerging alevins in rivers (Doucett et al. 1999*b*), and thus the relative contribution of the two forms to juvenile production can be calculated (Charles et al. 2004; Curry 2005). Consistent differences in stable carbon ($^{13}C/^{12}C$ or $\delta^{13}C$) and nitrogen ($^{15}N/^{14}N$ or $\delta^{15}N$) isotope ratios of primary producers in marine and freshwater environments are conserved in the body tissues of fishes that reside within these two environments (France 1995). As progeny of those fishes feed and grow in natal streams, they dilute their maternal isotope ratios with new biomass, eventually adopting the isotope ratios of resident fishes (Doucett et al. 1999*b*). However, if sampled early enough in life, offspring of the two forms can be identified (Curry 2005).

In Beadle Brook, New Brunswick, Canada, sea-run and resident brook trout (*Salvelinus fontinalis*) exist in sympatry below an impassable waterfall (Fig. 1). Earlier work conducted in 1999 using SIA found only a small contribution to overall production by sea-run trout (10%), despite a large number of adults known to be sea-run in the system (Curry 2005). This work, however, was based on a limited sample size (29 individuals below the impassable waterfall), was conducted over a short time later in the emergence period, and no measures of energy use (e.g., condition, lipid content) were taken, prompting further investigation in this system. A small contribution by sea-run fish to the overall juvenile population would have major management implications for this stock that is prized by anglers; therefore, we sought to re-evaluate in more depth the contribution of the two forms over space and time in the current study.

Our objectives in this study were threefold. First, we evaluated the utility of using SIA to distinguish between sea-run and resident trout offspring (Doucett et al. 1999*b*; Charles et al. 2004; Curry 2005) both upon emergence and when captured in the stream soon after emergence. Second, we determined if potential adaptive advantages were apparent in energy characteristics between progeny of the two forms. Our measures of fitness were body length and weight, condition, and elemental carbon to nitrogen ratios (C/N). %C and %N data are often provided alongside stable isotope data, and high-lipid tissues have higher C/N (McConnaughey and McRoy 1979), which makes C/N an index of lipid content. Our third and final objective was to assess the relative contribution of sea-run and resident females to juvenile production in the stream to compare with an earlier estimate that had indicated a low proportion of offspring of sea-run females (Curry 2005). All of these measures were

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conducted to determine the stability of the sea-run strategy in this system and to fill in gaps in our knowledge of the early life characteristics of sea-run and resident trout (Perry et al. 2005).

Materials and methods

Sample collection and preparation

Beadle Brook (46.63 N, 67.17 W) is a tributary of the Miramichi River in New Brunswick, Canada (Fig. 1). Sites were selected in the main stem of the brook and a tributary (north Beadle Brook) above and below a waterfall that is impassable to migratory fishes. A total of nine sites were selected to represent areas inhabited by resident fishes alone and those where sea-run and resident fishes existed in sympatry; areas were targeted where redds were observed in previous surveys.

Emergence traps were set on redds at a variety of sites in the brook above and below the waterfall (Chernoff and Curry 2007). Traps were set to capture progeny of suspected sea-run and resident fish, evaluated based on redd size (larger sea-run females construct larger redds than resident females; Curry and Noakes 1995). Traps were checked every 1–7 days (depending on emergence rates) and emergent fish removed. These fish were killed, total lengths were measured (TL, ± 1 mm), and the fish were transported on ice, oven dried, weighed to the nearest 0.0001 g (dry weight, DW), and ground to a homogenate for SIA. These fish will hereafter be referred to as trap fish.

To assess differences between sea-run and resident progeny after emergence, sampling was conducted using dip nets from the stream banks at several locations in the brook (sites indicated on Fig. 1) over the month following emergence (Curry et al. 1997). Fish were dipped from the stream (hereafter referred to as net fish), killed, total lengths were measured (±1 mm), and fish were transported on ice, oven dried, weighed to the nearest 0.0001 g, and ground to a fine homogenate for SIA. Whole-fish isotope ratios approximate those of white muscle (Pinnegar and Polunin 1999), the most commonly used tissue for SIA. For both trap fish and net fish, condition factor (K) was calculated as $DW \times TL^{-3} \times 10^5$ (Ricker 1975).

To link isotope ratios in fishes from the main stem and north branch of Beadle Brook with available food sources throughout the brook, benthic invertebrate samples were collected in D-nets at three sites in the main stem (B, D, G) and one site in the north branch (H) (Fig. 1). These samples were used to create expected equilibrium isotope ratios for trout following emergence and growth in the stream. Three taxa representing three different functional feeding groups (Merritt and Cummins 1996) were sampled from each site: Simuliidae (collector-filterers), Chironomidae (collector-gatherers), and Rhyacophilidae (predators). Samples were stored in ethanol prior to SIA.

Dried invertebrate and fish tissues were weighed into tin cups at ~0.2 mg for analysis of stable carbon and nitrogen isotopes. Samples were combusted in a Carlo Erba NC2500 elemental analyzer and transported in a helium stream via continuous-flow to a Thermo Finnigan Delta Plus mass spectrometer (Thermo, Bremen, Germany). Isotope ratios are expressed in delta notation (δ in permil, ‰) according to the formula: $\delta X = (R_{sample}/R_{standard} - 1) \times 1000$, where X is the heavier isotope (^{13}C or ^{15}N), R is the ratio of heavy to light isotope ($^{13}C/^{12}C$ or $^{15}N/^{14}N$), and standards are Vienna Peedee Belemnite carbonate (VPDB) and atmospheric nitrogen (AIR) for carbon and nitrogen, respectively. Samples were calibrated with International Atomic Energy Agency (IAEA) standards CH6, CH7, N1, and N2. A commercially available standard (acetanilide) had $\delta^{13}C = -33.5‰ \pm 0.2‰$ standard deviation (SD), $\delta^{15}N = -3.0‰ \pm 0.3‰$ SD, and $C/N = 6.88 \pm 0.21$ SD ($n = 48$). A single sample (emergent fry) was analyzed multiple times whenever samples were run and yielded $\delta^{13}C = -28.8‰ \pm 0.1‰$ SD, $\delta^{15}N = 11.0‰ \pm 0.2‰$ SD, and $C/N = 3.64 \pm 0.10$ SD ($n = 5$), and sample replicates within analytical runs had an average SD of 0.15‰ for $\delta^{13}C$, 0.19‰ for $\delta^{15}N$, and 0.11 for C/N.

Classification modeling

To classify sea-run and resident progeny captured in the stream (net fish) that had begun feeding and growing on available invertebrate prey, we used a simple isotope growth dilution model (Fry and Arnold 1982). This model can be used because the vast majority of isotopic change following a switch in diet by juvenile ectotherms is driven by growth (e.g., Fry and Arnold 1982; Vander Zanden et al. 1998). In the model, fish exponentially approach an equilibrium isotope ratio as they feed and grow in the stream.

For stable carbon, we used the formula

$$\delta^{13}C_t = \delta^{13}C_e + (\delta^{13}C_i - \delta^{13}C_e)(w_i/w_t)$$

where $\delta^{13}C_t$ is predicted $\delta^{13}C$ at time t, $\delta^{13}C_e$ is the $\delta^{13}C$ when in equilibrium with the new diet, $\delta^{13}C_i$ is the initial $\delta^{13}C$, and w_i and w_t are initial weight and weight at time t, respectively. $\delta^{13}C_e$ was determined by measuring isotope ratios in young-of-the-year (YOY) trout and in a diet on which they were reared from emergence in the lab (Chernoff and Curry 2007). These measurements yielded diet–tissue ^{13}C fractionation (muscle $\delta^{13}C$–diet $\delta^{13}C$) of 2.3‰. Because invertebrates in Beadle Brook had a mean $\delta^{13}C$ of –31.4‰, our expected $\delta^{13}C$ for a YOY trout in equilibrium with its diet in the stream ($\delta^{13}C_e$) was –29.1‰.

To capture the upper and lower bounds of the initial data in this equation ($\delta^{13}C_i$), we plotted $\delta^{13}C$ of trap fish vs. DW (Fig. 2). We then developed two equations for each morph that would include all predicted trap fish data within its upper and lower limits as the fish approached equilibrium with the new diet. We superimposed these predicted equilibration curves over our net fish of unknown parentage (Fig. 3). Those individuals whose $\delta^{13}C$ fell within the defined bounds of the different morphs were classified accordingly.

Classification using $\delta^{15}N$ was done following the same procedure as above but by substituting $\delta^{15}N$ for $\delta^{13}C$ in all cases. $\delta^{15}N_e$, also based on measurements made on trout fry reared in the lab, was 9.1‰. This was calculated from invertebrate isotope ratios in Beadle Brook ($\delta^{15}N = 5.6‰$) and diet–tissue fractionation in the lab of 3.5‰. If the two isotopes yielded different classification (e.g., $\delta^{13}C$ indicated a resident progeny and $\delta^{15}N$ indicated a sea-run progeny), the fish was excluded from the analysis. This occurred in only

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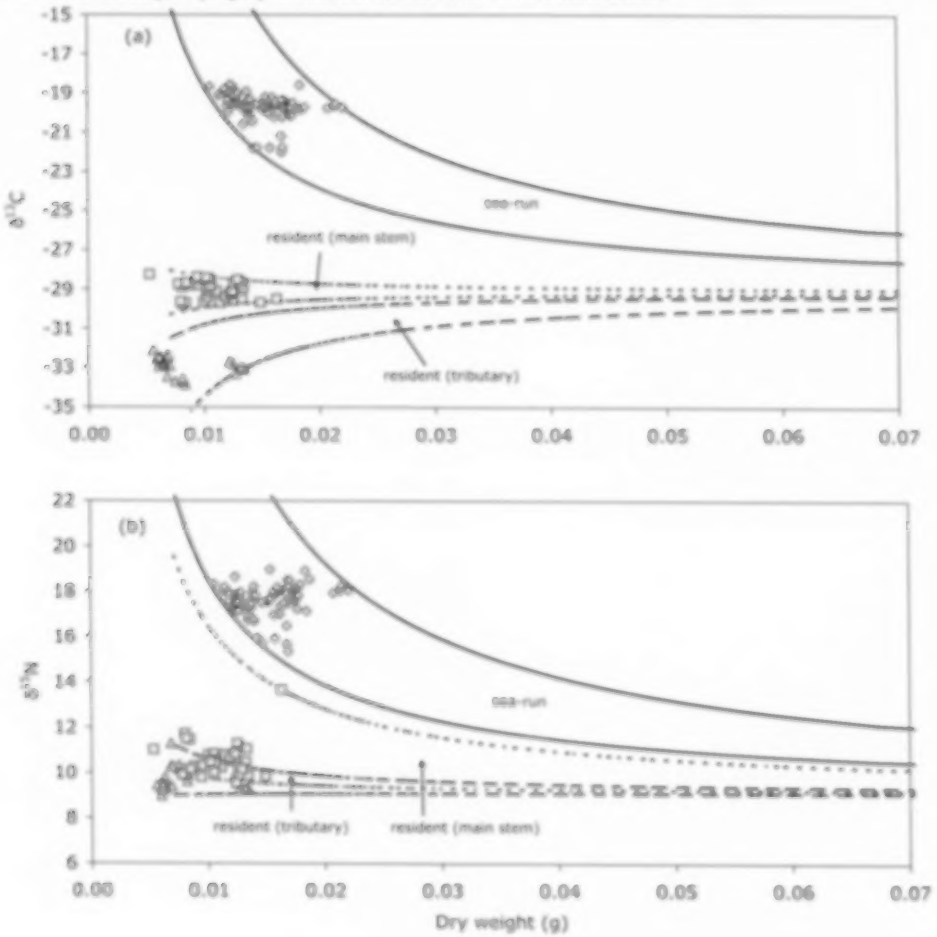
Fig. 2. Stable carbon (*a*) and nitrogen (*b*) isotope dilution curves for brook trout (*Salvelinus fontinalis*) progeny in Beadle Brook, New Brunswick, Canada, as they come into isotopic equilibrium with invertebrates from the stream. Curves were generated to capture all observed data from emergence traps for progeny from the three sources (diamonds, progeny of sea-run females; squares, progeny of resident females from the main stem; triangles, progeny of resident females from north Beadle Brook).



12 cases (of 196 total net fish), indicating good agreement between $\delta^{13}C$ and $\delta^{15}N$.

Statistics

For trap fish, unique stable isotope ratios allowed separation of three groups of progeny: sea-run, mainstem resident, and tributary resident. We used single factor analysis of variance (ANOVA) to test for differences in TL, DW, *K*, and C/N in progeny of these three groups. For net fish, owing to overlapping $\delta^{15}N$ values between the two resident populations in our model (Fig. 2), our isotope dilution model only allowed separation of two groups: sea-run progeny and resident progeny. We used single factor ANOVA to test for differences in TL, DW, *K*, and C/N in progeny of these two groups. Following classification of YOY captured in nets in the stream, we were able to estimate the proportion of the total catch from sea-run and resident females (residents included subpopulations in the main stem and tributary).

Results

A total of 127 YOY trap fish were captured. These trap fish separated into three clearly distinct groups based on $\delta^{13}C$ and $\delta^{15}N$ (Fig. 4). Two groups of residents were apparent: one in the main stem of Beadle Brook ($\delta^{13}C$ = –29.0‰ ± 0.4‰ SD, $\delta^{15}N$ = 10.6‰ ± 0.8‰ SD, n = 31) and one in the north branch of the brook ($\delta^{13}C$ = –32.9‰ ± 0.5‰ SD, $\delta^{15}N$ = 9.7‰ ± 0.5‰ SD, n = 21). These two groups had isotope ratios that corresponded with invertebrate prey available in the two habitats (main-stem invertebrates: $\delta^{13}C$ = –30.6‰ ± 1.6‰ SD, $\delta^{15}N$ = 5.4‰ ± 1.5‰ SD, n = 11 pooled samples; north branch invertebrates: $\delta^{13}C$ = –34.0‰ ± 1.7‰ SD, $\delta^{15}N$ = 6.1‰ ± 0.4‰ SD, n = 3 pooled samples). Progeny of sea-run females were highly enriched in ^{13}C and ^{15}N relative to the two resident groups (sea-run $\delta^{13}C$ = –19.8‰ ± 0.7‰ SD, $\delta^{15}N$ = 17.6‰ ± 0.7‰ SD, n = 63; Fig. 4). Isotope ratios of sea-run progeny indicated a maternal diet of

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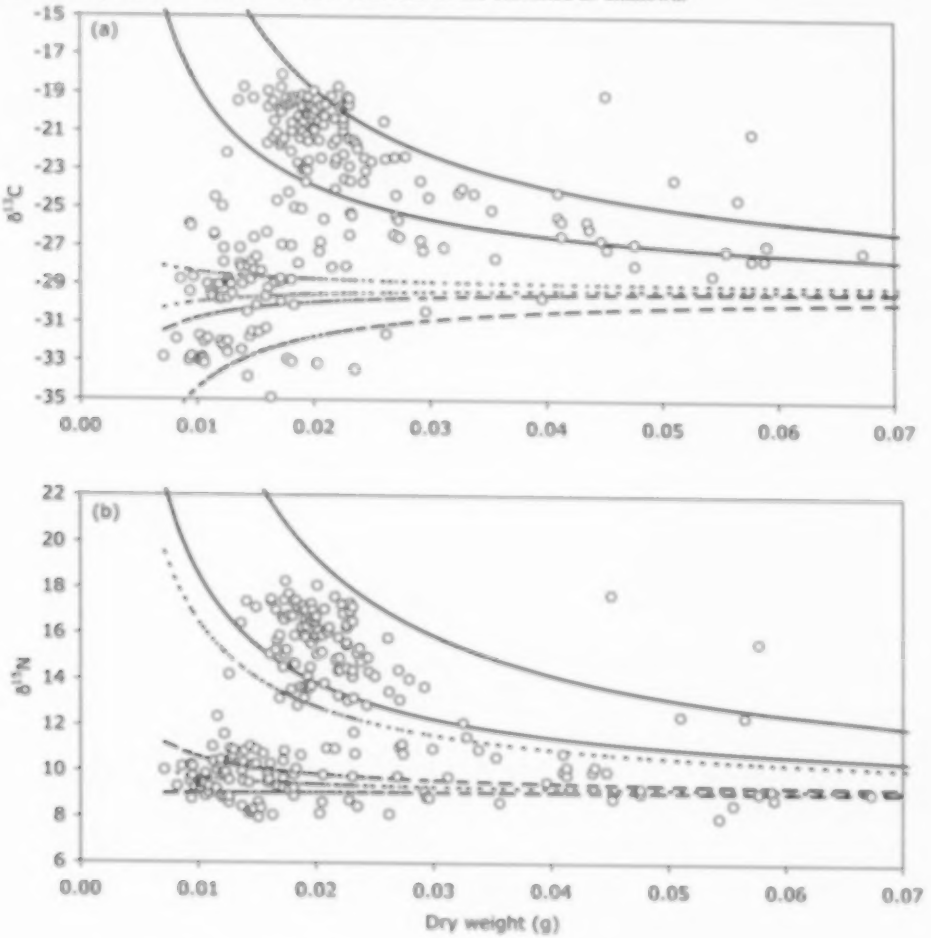
Fig. 3. Stable carbon (*a*) and nitrogen (*b*) isotope ratios vs. dry weight for brook trout (*Salvelinus fontinalis*) progeny captured in dip nets in Beadle Brook, New Brunswick, Canada. Curves were generated to capture all observed data from emergence traps for progeny from the three sources (solid line, progeny of sea-run females; stippled line, progeny of resident females from the main stem; dashed line, progeny of resident females from north Beadle Brook), and all fish falling inside the bounds of one curve are classified as sea-run or resident progeny accordingly. Fish with isotope ratios outside the bounds of all curves are classified as unknown.



invertebrates and small fishes in the Miramichi estuary, based on data obtained from Pastershank (2001) (Fig. 4).

There were differences in DW values ($F = 86.05$, $p < 0.001$), total lengths ($F = 107.88$, $p < 0.001$), and C/N ($F = 4.12$, $p = 0.018$) among progeny of different morphs captured in emergence traps, but no differences in K ($F = 0.51$, $p = 0.600$). Residents from the north branch of the brook were significantly shorter and lighter than residents from the main stem, and progeny of sea-run females were longer and heavier and had higher C/N than both resident groups (Table 1).

A total of 239 YOY net fish were captured from the stream banks, and 184 were successfully classified as progeny of sea-run or resident fish using our model. Following classification, we were able to test for differences in body size, condition, and C/N between net fish progeny of sea-run and resident females. Sea-run progeny were significantly longer, heavier, had higher C/N than residents ($F = 31.945$, $p < 0.001$) and surprisingly also had higher K values ($F = 5.890$, $p = 0.016$) (Table 2).

Sea-run progeny made up 5%–100% of the catch at sites below the impassable waterfall, with an average contribution of 56% (Fig. 5). Below the waterfall, residents made up 0%–50% of the catch, with an average of 19%. The remainder (25%) consisted of unknown individuals that we were unable to classify. Above the waterfall, no progeny were mistakenly classified as sea-run (Fig. 5), with all individuals classified as resident or unknown.

Discussion

Our results indicate that progeny of sea-run females are afforded potential adaptive advantages during their early stages of development through the transfer of energy resources acquired at sea. In the Beadle Brook system, sea-run progeny dominate the population below the waterfall where

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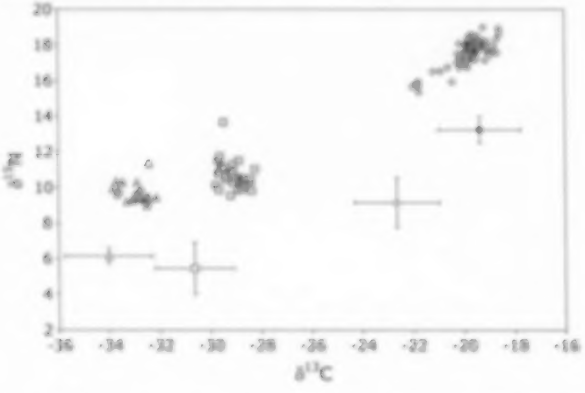
Fig. 4. Dual isotope biplot ($\delta^{13}C$ and $\delta^{15}N$) of emerging brook trout (*Salvelinus fontinalis*) progeny in Beadle Brook, New Brunswick, Canada. Triangles indicate progeny of resident trout (shaded) and invertebrates (open; mean ± standard deviation, SD) from north Beadle Brook, squares are progeny of resident trout (shaded) and invertebrates (open; mean ± SD) from the mainstem Beadle Brook, and diamonds are progeny of sea-run trout (open) and small fishes (solid; mean ± SD) and invertebrates (open; mean ± SD) from the Miramichi estuary. Data for the Miramichi estuary are modified from Pastershank (2001).



the two forms exist in sympatry. These results suggest that for some environments (e.g., waters with appropriate habitat to support larger-bodied sea-run fish), the sea-run strategy is favoured during early life either as a heritable characteristic distinct from the resident strategy (Chernoff and Curry 2007) or as a plastic response to environmental conditions that enhances fitness at the population level (Olsson et al. 2006).

Progeny of sea-run fish were larger and had higher *K* values than progeny of resident fish despite having a later average emergence date (Chernoff 2006). Given the lack of difference in *K* between resident and sea-run progeny at emergence (i.e., trap fish), it was surprising that individuals captured from the stream margins (i.e., net fish) showed differences in *K*. Potentially, the conversion of more abundant lipid reserves as indicated by higher C/N (McConnaughey and McRoy 1979) is a possible key factor leading to increased *K* over time during the first summer for sea-run progeny. Sea-run progeny showed an increase in *K* from 0.124 to 0.138 from emergence to their capture as free-swimming alevins or fry that occurred in concert with a reduction in C/N from 4.13 to 3.87. Resident progeny also showed a reduction in C/N between samples, but did not exhibit a parallel rise in *K*. In a controlled laboratory experiment, Chernoff and Curry (2007) also observed greater *K* over the first summer in sea-run progeny from Beadle Brook, yet differences in body size were not apparent in another population with sea-run fish (Doucett et al. 1999*b*). Differences in *K* and body size in the former study persisted until late August. Enhanced body size and *K* in sea-run progeny could have population-level consequences because larger stream fishes with more abundant body reserves are more likely to survive the first winter of life (Hutchings 1991; Finstad et al. 2004; Bystrom et al. 2006). Also, the greater energy reserves carried by progeny of sea-run females could allow easier access to faster currents in streams, which may in turn necessitate greater food consumption and increase the likelihood of a future migration to sea (Morinville and Rasmussen 2003; 2006*b*). If the propensity to migrate to sea is heritable, then increased survival of sea-run juveniles could lead to a higher proportion of sea-run adults in the population (i.e., if there are habitats to support large sea-run adults). Alternatively, migration to sea may be plastic in response to changing environmental conditions, including factors such as fishing mortality (Hutchings 2005) (i.e., recreational fishers of sea-run brook trout) and conditions in the stream (e.g., food availability, Olsson et al. 2006).

Based on the dramatic separation in $\delta^{13}C$ and $\delta^{15}N$ between sea-run progeny and our most ^{13}C- and ^{15}N-enriched resident progeny, it appears that brook trout in this population are capital breeders because they use resources for reproduction acquired far away from the breeding grounds (Bonnet et al. 1998; Houston et al. 2007). Had this sea-run population consisted of income breeders, we would have observed a continuous isotopic gradient from the resident forms up to the most elevated $\delta^{13}C$ and $\delta^{15}N$, because sea-run females feeding in fresh water would dilute the marine isotope ratios in their egg tissue. Tagging studies with Beadle Brook sea-run adults have revealed that some fish leave the stream after spawning (November–December), arriving and wintering at the head of tide area, make some forays into the estuary for a few weeks in spring (April–May), and return to Beadle Brook by June (R.A. Curry, unpublished data; see also van de Sande 2004). Stable carbon ratios in sea-run progeny (–20‰) in the current study were similar to those for adult brook trout feeding in an estuarine fiord (Morinville and Rasmussen 2006*a*), but stable nitrogen ratios were enriched in ^{15}N ($\delta^{15}N$ = 18‰) relative to sea-run trout from the same study (~12‰, Morinville and Rasmussen 2006*a*). This suggests considerable piscivory of marine species by Beadle Brook females, consistent with that observed for fish above 25 cm in length (Morinville and Rasmussen 2006*a*). When and where they forage remains somewhat unresolved. During the winter period, sea-run adults rarely move (Curry et al. 2002; Curry et al. 2006), and foraging is likely greatly reduced at low temperatures (Koskela et al. 1997). Regardless, the Miramichi River estuary where the adults are located has an abundance of estuarine prey species (e.g., mummichog (*Fundulus heteroclitus*) and Atlantic tomcod (*Microgadus tomcod*)). In late winter and early spring as brook trout become active, they could be consuming anadromous rainbow smelt (*Osmerus mordax*) and alewife (*Alosa pseudoharengus*) that move from the sea into the river as the brook trout begin returning upstream. This short period may be the critical time for growth and gonad development in sea-run brook trout (Hutchings 1991; Tyler and Sumpter 1996).

An alternative explanation of the high $\delta^{15}N$ in sea-run progeny observed in Beadle Brook may be the preferential incorporation of ^{15}N over ^{14}N into egg tissue because of the predominance of particular amino acids, or it may reflect the recycling of N within the developing embryos that could also lead to ^{15}N enrichment (Hobson et al. 1993). Other studies have shown evidence of slight ^{15}N enrichment in egg and alevin tissue relative to that of adult muscle or fin

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Table 1. Total length (TL, mm), dry weight (DW, g), condition (K, g·cm^{-3} × 10^5), and C/N of sea-run and resident brook trout (*Salvelinus fontinalis*) captured in emergence traps in Beadle Brook, New Brunswick, Canada.

YOY origin	TL (mm)	DW (g)	K (g·cm^{-3} × 10^5)	C/N	n
Sea run	23.4±0.2^a	0.0155±0.0003^a	0.124±0.004^a	4.13±0.04^a	73
Resident					
Mainstem	20.4±0.2^b	0.0106±0.0004^b	0.128±0.007^a	3.98±0.05^b	32
North	19.0±0.3^c	0.0082±0.0005^c	0.118±0.006^a	3.95±0.06^b	22

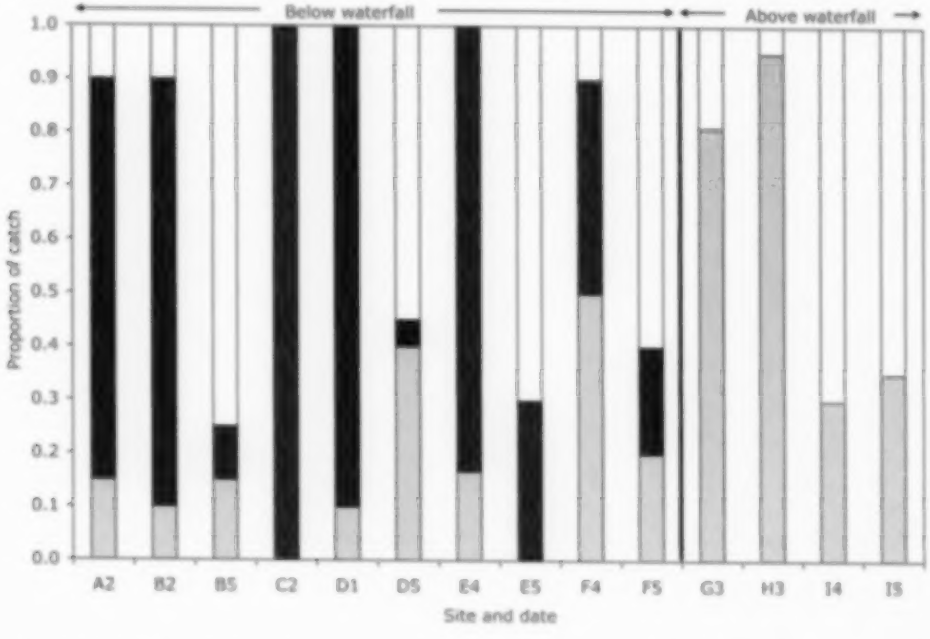
Note: Different letters indicate significant differences at $\alpha = 0.05$ (analysis of variance, ANOVA). Residents are classified in two separate groups: main stem and north Beadle Brook. YOY, young of the year.

Table 2. Total length (TL, mm), dry weight (DW, g), condition (K, g·cm^{-3} × 10^5), and C/N of sea-run and resident brook trout (*Salvelinus fontinalis*) captured in dip nets in Beadle Brook, New Brunswick, Canada.

YOY origin	TL (mm)	DW (g)	K (g·cm^{-3} × 10^5)	C/N	n
Sea run	25.0±0.3^a	0.0221±0.0008^a	0.138±0.002^a	3.87±0.02^a	101
Resident	23.3±0.5^b	0.0165±0.0010^b	0.129±0.003^b	3.74±0.02^b	83

Note: Different letters indicate significant differences at $\alpha = 0.05$ (analysis of variance, ANOVA). YOY, young of the year.

Fig. 5. Estimated proportion of catch for progeny of sea-run (solid bars), resident (shaded bars), and unknown (open bars) female brook trout (*Salvelinus fontinalis*) in Beadle Brook, New Brunswick, Canada. Site codes are as in Fig. 1. Date codes are as follows: 1 = 27 April, 2 = 28 April, 3 = 29 April, 4 = 3 May, 5 = 17 May.



tissue (~1‰ in slimy sculpin (*Cottus cognatus*), Jardine et al. 2005*b*; ~2‰ in coho salmon (*Oncorhynchus kisutch*), Bilby et al. 1996); while these differences are small, they could explain the difference in $\delta^{15}N$ between sea-run progeny (mean $\delta^{15}N$ = 18‰) and potential fish prey from the Miramichi estuary ($\delta^{15}N$ ~ 13‰, Pastershank 2001) after accounting for trophic fractionation.

We predict that our classification of sea-run and resident fish was conservative for net fish. For example, individuals that had high $\delta^{13}C$ and $\delta^{15}N$ for a given body size are likely sea-run progeny, but because they fell outside the bounds predicted for sea-run progeny coming into equilibrium with a stream diet, they remained classified as unknown. Similarly, those fish with low $\delta^{13}C$ and $\delta^{15}N$ for a given body size were also classified as unknown despite likely being offspring of resident females. Capturing fish immediately after emergence almost guarantees 100% classification using stable isotopes, as observed for trap fish in the current study

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(providing a high degree of resolution with residents separating further into two groups) and for emerging brown trout (*Salmo trutta*) in Brittany, France (Charles et al. 2004). For net fish, in comparison with earlier methods using otoliths to classify progeny of sea-run and resident salmonids (e.g., Rieman et al. 1994), our method successfully classified ~75% of all fish captured swimming in the stream, which was slightly higher than that reported for otoliths (~65%, Rieman et al. 1994). Using otoliths to determine maternal origin is cumbersome analytically and requires validation of source strontium to calcium ratios that are used to identify rearing areas (Rieman et al. 1994). SIA, meanwhile, has advanced analytically to allow rapid sample preparation and throughput, is now available commercially, and has a large body of literature documenting baseline isotope ratios across a wide range of aquatic habitat types (Fry and Sherr 1984; France 1995). The technique of separating sea-run and resident fish in this study could be applied to many ecological and evolutionary questions, such as the use by sea-run juveniles of faster currents in rivers because of higher energy demands and food consumption rates (Morinville and Rasmussen 2003, 2006*b*). Indeed, our sample collection techniques (netting fish near the stream banks) may have underestimated the number of sea-run progeny below the waterfall if they inhabited faster currents in the centre of the stream.

The major limitation of the isotope approach remains the loss of the maternal signal during growth in the stream (Curry 2005) that creates a window of opportunity wherein progeny of resident and sea-run females can be distinguished, after which the differences are no longer apparent (Curry 2005). This window of opportunity is limited because of rapid growth rates of fish in early life, on the order of 1–2 months after emergence, corresponding to a relatively small body size (e.g., ~30 mm fork length) and may occur even faster in other species (e.g., ~2 weeks after emergence and ~20 mm total length in smallmouth bass (*Micropterus dolomieu*); Vander Zanden et al. 1998).

The high proportion of sea-run progeny making up the catch below the impassable waterfall (average = 56%) is far higher than that observed in a prior study in this system (10%, Curry 2005). The higher contribution we observed would be predicted based on the consistently large numbers of sea-run females moving in and out of the lower reaches each year. The prior estimate of 10% sea-run progeny for this system was most probably an artifact of limited sampling in space and time or an overestimate of the resident contribution due to sampling after sea-run progeny had grown enough to come into equilibrium with their new diet of stream insects. Curry (2005) hypothesized that sea-run progeny could have dispersed downstream at the time of sampling, but with the decreasing ability to resolve sea-run from resident progeny over time, there was no strong evidence of increased proportions of resident progeny later in the emergence period in the present study. However, decreases in the proportion of sea-run progeny between sampling dates (e.g., 80% to 10% at site B from 28 April to 17 May, 90% to 5% at site D from 27 April to 17 May) were more dramatic than those observed in resident progeny, suggesting possible downstream dispersal of sea-run progeny.

The use of SIA to classify progeny as sea-run or resident continues to improve our knowledge of reproductive and early life history tactics and strategies for sympatric populations. Given the potential adaptive advantages of larger body size, higher condition, and greater lipid reserves, it is perhaps not surprising that the scope for growth is much greater in sea-run progeny (Morinville and Rasmussen 2003; Chernoff and Curry 2007). However, given that genetic differentiation between sea-run and resident salmonid forms is relatively rare (Perry et al. 2005; Charles et al. 2006) and absent in this particular population (Rogers and Curry 2004), it is unknown whether the decision to migrate is indeed heritable. The stable isotope approach therefore tells us little about the future life history strategy of progeny of the two forms, and other techniques (e.g., back-calculated growth rates, genetic analyses) will be required to address those questions. Migration studies of progeny of sea-run and resident females reared in seminatural enclosures hold promise in providing links between the life history strategy adopted by the parent and that of their progeny. Regardless, the sea-run strategy appears to impart adaptive advantages for progeny in the first year of life, both in terms of numbers of offspring produced (Thériault et al. 2007*a*) and the energy characteristics of those progeny. Whether this represents divergent selection of a heritable characteristic, a plastic response to balance the risks associated with going to sea, or, most likely, a combination of both processes (Thériault et al. 2007*b*), there are important implications for the survival and long-term stability of individuals and populations.

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The cultural eutrophication of Lac la Biche, Alberta, Canada: a paleoecological study

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Abstract: A multiproxy paleoecological investigation of Lac la Biche, a large boreal lake in northeastern Alberta, Canada, revealed that the lake was eutrophic before European settlement but has undergone additional cultural eutrophication in the past 30 to 50 years. Annual fluxes to sediments of phosphorus, nitrogen, carbon, and inorganic sediments have increased with time. A declining N–P ratio has increasingly favored nitrogen-fixing cyanobacteria. Increased deposition of microbial pigments and diatom frustules and a recent shift in diatom species also indicate increasing eutrophication. Biogenic silica increased with time, but there is no evidence of a near-surface decline that would indicate silica limitation. Stable isotopes suggest that an increasing proportion of carbon deposited in sediments is of in-lake origin, indicating increased productivity. In the basin nearest the town of Lac La Biche, an increase in $\delta^{15}N$ followed the construction of the sewage treatment plant, but more recently, decreased $\delta^{15}N$ in both basins suggests that nitrogen fixation has become a more important source of nitrogen. Despite documented damage to the fishery of the lake, zooplankton fossils do not show evidence of a strong trophic cascade. The study illustrates the power of a multiproxy approach in obtaining reliable paleolimnological conclusions.

Résumé : Une étude paléoécologique avec plusieurs variables de substitution au lac La Biche, un grand lac boréal du nord-est de l'Alberta, Canada, montre que le lac était eutrophe avant l'arrivée des européens, mais qu'il a subi une eutrophisation culturelle supplémentaire au cours des 30 à 50 dernières années. Les apports annuels aux sédiments de phosphore, d'azote, de carbone et de sédiments inorganiques se sont accrus au cours des années. Un rapport N–P en déclin a de plus en plus favorisé les cyanobactéries fixatrices d'azote. Les dépôts croissants de pigments microbiens et de frustules de diatomées, de même qu'un changement récent dans les espèces de diatomées, indiquent une intensification de l'eutrophisation. Il y a une augmentation dans le temps de la silice biogène, mais aucune indication d'un déclin près de la surface qui pourrait signaler une limitation de la silice. Les analyses d'isotopes stables laissent croire qu'une proportion croissante du carbone déposé dans les sédiments provient du lac lui-même, ce qui indique une productivité accrue. Dans le bassin versant le plus près de la ville de Lac-La-Biche, une augmentation de $\delta^{15}N$ a suivi la construction d'une usine de traitement des eaux usées; plus récemment, une réduction de $\delta^{15}N$ dans les deux bassins fait penser que la fixation d'azote est devenue une source plus importante d'azote. Malgré les données sur les dommages causés aux pêches dans le lac, les fossiles du zooplancton n'appuient pas l'existence d'une importante cascade trophique. Notre étude est un exemple du potentiel des méthodes à nombreuses variables de substitution pour l'obtention de conclusions paléolimnologiques fiables.

[Traduit par la Rédaction]

Introduction

Lakes of the prairie provinces generally occupy catchments rich in phosphorus, so that they tend to be mesotrophic or mildly eutrophic even without human interference (Prepas and Trew 1983). Iron concentrations in the lakes are usually low, even under anoxic conditions. As a result, periodic anoxia causes remobilization of phosphorus from sediments. Similar conditions have been observed to facilitate phosphorus release from sediments in other shallow calcareous lakes (Stauffer and Armstrong 1984; Stauffer 1985). This internal loading can be 50% to 90% of the total phosphorus loading to the lake (Prepas and Vickery 1984; Mitchell 2001). Such releases occur both under winter ice,

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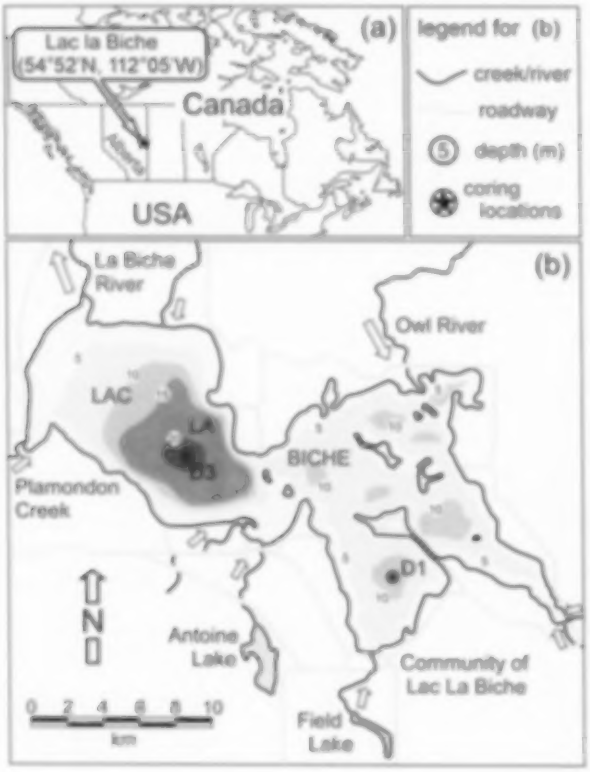
Fig. 1. (*a*) Map showing the location of Lac la Biche in western Canada. (*b*) Map of Lac la Biche showing the location of the town by the same name, the inflows and outflows of the lake, rough morphometry, and other features. D1 and D3 are the locations of coring sites at the deepest spots in the basin nearest the town and the main basin of the lake, respectively. Shading represents depth in 5 m intervals, where the lightest shading represents 5 m and the darkest represents 20 m.



contributing to the magnitude of the spring algal bloom, and in midsummer, when they help to maintain high algal populations. During the latter period, there is usually a scarcity of inorganic nitrogen, favoring bloom-forming species of nitrogen-fixing cyanobacteria (Prepas and Trimbee 1988; D.W. Schindler, unpublished data).

The lakes are generally shallow and usually well mixed by the wind. However, during periods of low wind velocity, transient thermoclines develop rapidly. Anoxia develops quickly in the small hypolimnions, and in the absence of iron, large quantities of phosphorus are released to overlying water (Schindler and Comita 1972; D.W. Schindler, unpublished data). Such thermoclines form and break periodically throughout the summer season, allowing phosphorus in the euphotic zone to be replenished periodically, depending on weather conditions. The high variability in seasonal weather thus complicates the prediction of phosphorus concentrations and algal blooms in polymictic lakes. Stauffer and Armstrong (1984) concluded that similar polymictic conditions and periodic release of phosphorus from sediments were the reasons why Shagawa Lake, Minnesota, did not respond quickly to reductions in external nutrient loading.

Table 1. Characteristics of Lac la Biche.

Elevation (m)	543.84
Surface area (km^2)	234
Volume (m^3)	1960×10^6
Maximum depth (m)	21.3
Mean depth (m)	8.4
Shoreline length (km)	172
Mean annual lake evaporation (mm)	702
Mean annual precipitation (mm)	524
Mean residence time (years)	7
Control structure	None
Terrestrial drainage (km^2)	4040
Total nitrogen D1, D3 ($mg \cdot m^{-3}$)	860, 846
Total dissolved nitrogen D1, D3 ($mg \cdot m^{-3}$)	695, 736
Total phosphorus D1, D3 ($mg \cdot m^{-3}$)	97, 68
Total dissolved phosphorus D1, D3 ($mg \cdot m^{-3}$)	73, 51
Chlorophyll *a* D1, D3 ($mg \cdot m^{-3}$)	16, 10

Note: Modified from Mitchell and Prepas (1990). Nutrient and chlorophyll *a* concentrations are simple means of ice-free season data collected between 2003 and 2005 (D.W. Schindler, unpublished data).

Lac la Biche (Lake of the Red Deer, a translation of Waskisew Sagihaygan, the Cree name Elk Lake; Fig. 1) is a large (234 km^2), shallow (mean depth 8.4 m, maximum depth 22 m) lake in northeastern Alberta. The lake is at a strategic portage between the Churchill and Athabasca–Mackenzie river systems and therefore has probably been occupied by aboriginal people for thousands of years. Key characteristics of the lake are given in Table 1.

For the past few decades, lakeshore residents have complained increasingly about poor water quality in the summer. There is a perception that nuisance blooms of cyanobacteria have intensified. A provincial study of the lake in 1990 concluded that there had been no deterioration in water quality, but this study was based on scarce data. In 2002, Lakeland County officials approached us to study the lake. Because of the shortage of historical data, we chose paleolimnological methods to assess past changes in water quality.

Lac la Biche exemplifies many of the lakes in the southern boreal and prairie parkland areas of Alberta in several ways, including the polymixis and low iron described above. Like other lakes in the area, its catchment has been subjected to extensive land clearing and agricultural activities in the past half century. A growing urban population in an affluent province has caused rapidly increasing concentrations of summer homes on the lakeshores.

Few of the lakes have been subjected to monitoring that is intense enough to reliably separate long-term trends in water quality from interannual variation. As a result, paleoecology could potentially provide useful information on the history of changes to many lakes of the region.

A brief history of Lac la Biche

The first known European settlement in the watershed was a Northwest Company trading post, Red Deer's Lake House, established on the southern shore of the lake by David Thompson in 1798. A Hudson's Bay Company (HBC) post, Greenwich House, the first HBC post beyond

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Rupert's Land, was established the following year by Peter Fidler. The Portage la Biche was the gateway to the southern Athabasca country from 1799–1824 and a passage to the Pacific coast from 1811–1824.

Métis settlements remained on the lake, and the Lac la Biche Mission on the shore of the lake was a popular gathering spot for religious or social events. A Cree settlement, the Beaver Lake First Nation, also has remained in the immediate area. There are several other aboriginal and Métis settlements in the region. The mission, now a historic site, still stands on the south shore of the lake.

The fishery of this productive lake was already heavily exploited in the 19th and 20th centuries (see supplementary material).[2] By the end of the 20th century, the walleye (*Sander vitreus*) fishery had collapsed, and other fisheries are under stringent regulations.

Clearing of the watershed for agriculture began in the late 19th century and accelerated after World War II as modern tractors became available. In the mid-20th century, when reliable automobiles and improved roads resulted in increased tourism from populous cities in southern Alberta, the town began to grow. The population of the town of Lac La Biche in 2006 was listed as 2758, but the community is growing rapidly as the result of increasing recreational activity and development in the nearby Alberta Oil Sands. A small (300 people), French-speaking community, Plamondon, the only other community on the lake, is nestled in the valley of Plamondon Creek, which enters Lac la Biche at the western end of the lake.

Sewage began to be discharged into the lake in 1951 as the town of Lac la Biche constructed its first waste treatment plant. In 1983, the residents, who also drew their drinking water from the lake, decided that this posed too much of a threat to water quality. Sewage was diverted, discharging to Field Lake, upstream of Lac la Biche. Subsequent studies show that much of the sewage drains back to Lac la Biche via Red Deer Brook. Provincial agencies referred to the sewage as "growth-enhancing effluent" and regarded Field Lake as of marginal importance. The treatment plant was upgraded in 1989 but continued to discharge to Field Lake. A plan for a new treatment plant to remove nutrients or divert sewage from Field Lake is under discussion. A golf course along the southeastern shore of the lake is also a possible source of nutrients.

With continuous improvement in roads and increased population since the mid-20th century, recreational cottages have increased in number, largely along the southern shores of the lake. About 50% of the immediate catchment of the lake has been cleared for agriculture. Most is used as hayland or pasture, and there is little application of fertilizer.

By the early 1990s, residents of the area increasingly complained about water quality, particularly surface algal blooms and decreased clarity. Swimmer's itch, high fecal coliform counts near some beaches, and beach closures also became more common.

Materials and methods

On 1 February 2003, using a Glew gravity corer, we took short cores from the deepest locations in different basins of the lake. Subsequent limnological work showed that there was considerable similarity in the water quality at the three easternmost coring sites in the eastern part of the lake. Here we interpret the results of cores taken at the deepest points in the main (western) basin of the lake at site D3 (54°50.049′N, 112°09.727′W) and in the basin nearest the town of Lac La Biche, which is the most eutrophic of the lake's basins, at D1 (54°49.920′N, 111°55.838′W; Fig. 1). Cores were sliced at 0.5–1 cm intervals, dated by lead-210 (^{210}Pb) and analyzed for nutrients, algal fossils (diatoms and chrysophytes), dry weight, microbial pigments, biogenic silica, stable isotopes, and cladoceran remains. Sections were preserved for analysis in Whirl-Pak® bags and refrigerated. The depths at which cores were taken were 22 m at D3 and 9 m at D1. The cores analyzed were 45 cm (D3) and 50 cm (D1) in length, which included the entire period of recent changes in lake. We used methods that are widely accepted by the paleolimnological community for diagnosing changes in the trophic state of lakes (Douglas and Smol 1999; Riedinger-Whitmore et al. 2005; Schelske et al. 2006).

Dating using ^{210}Pb analysis by gamma spectrometry was done at the University of Ottawa. Loss on drying, loss on ignition, total phosphorus, total nitrogen, biogenic silica, and stable isotope analyses were conducted in the Limnology Laboratory in the Department of Biological Sciences at the University of Alberta. Diatom analyses were performed at the Department of Earth and Atmospheric Sciences, and pigments were analyzed in the Freshwater Biodiversity Laboratory, both at the University of Alberta (Edmonton, Alberta).

Diatom slides were prepared from 25 samples in core D1 and from 27 samples in core D3, with greater sampling density towards the surface. In each case, 200 µg of freeze-dried sediment was boiled progressively in 10% and 30% H_2O_2. Cleaned and diluted slurries were spiked with a known quantity of *Eucalyptus* pollen grains to enable quantitative assessments of diatom concentrations (Wolfe 1997). Diatom sums ranged from 300 to 500 valves per sample. Chrysophyte stomatocysts were also counted, but no effort was made to differentiate them taxonomically. Preservation was poor in all but the upper 7 cm of the D1 core but was good for the entire length of core D3. Diatom taxonomy was assisted by regional western Canadian floras (Cumming et al. 1995; Moser et al. 2004), augmented by other sources (Patrick and Reimer 1966, 1975; Krammer and Lange-Bertalot 1986–1991). Diagrams of diatom and chrysophyte stomatocyst concentrations alongside sediment biogenic silica and of down-core assemblage composition expressed as frequencies relative to the sum counted are shown.

Microbial pigment concentrations were quantified using a standard reverse-phase high-pressure liquid chromatographic technique (HPLC; Vinebrooke et al. 1998). Pigments were

[2] Supplementary data for this article are available on the journal Web site (http://cjfas.nrc.ca) or may be purchased from the Depository of Unpublished Data, Document Delivery, CISTI, National Research Council Canada, Building M-55, 1200 Montreal Road, Ottawa, ON K1A 0R6, Canada. DUD 3825. For more information on obtaining material refer to http://cisti-icist.nrc-cnrc.gc.ca/cms/unpub_e.html.

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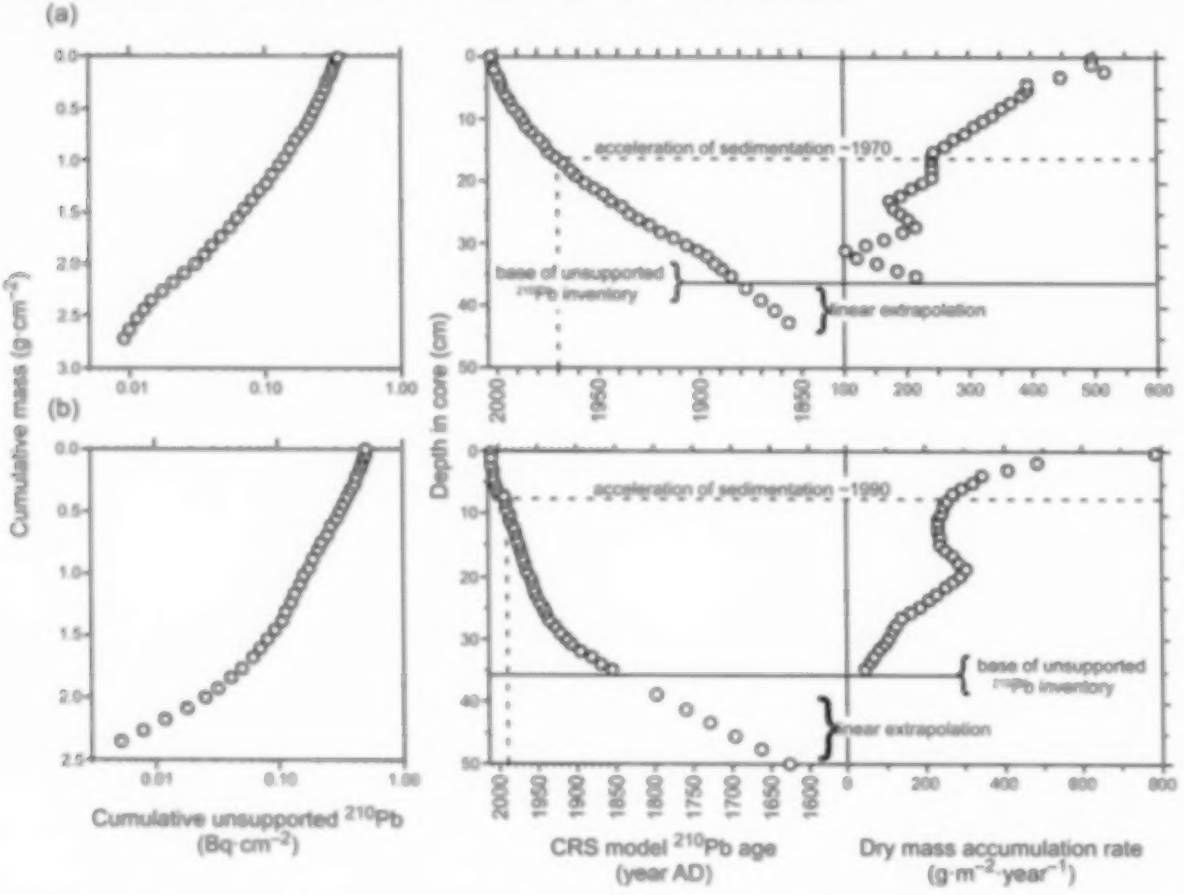
Fig. 2. Cumulative unsupported ^{210}Pb (dates based on the constant rate of supply (CRS) model) and dry mass accumulation of sediment at (*a*) site D1 and (*b*) site D3 in Lac la Biche. Cores D1 and D3 were 42 and 50 cm long, respectively.



first extracted from freeze-dried sediments using an acetone–methanol solution. Extracts were then filtered (0.2 µm pore nylon), dried under N_2, and reconstituted using a precise volume of injection solution. Chromatographic separation was performed with an Agilent 1100 Series HPLC (Agilent Technologies Canada Inc., Calgary, Alberta) equipped with a Varian Microsorb 100 C18, and pigment was detected using in-line diode array and fluorescence detectors. Pigment concentrations were quantified via calibration equations and an electronic spectral library of standards purchased from DHI Water and Environment (Agern Alle 5, DK-2970 Hoersholm, Denmark).

Sediments for biogenic silica (BSi) analysis were predigested according to Mortlock and Froelich (1989) and analyzed using the wet alkaline method of Conley and Schelske (2001). Briefly, 30 mg of sediment was predigested with 10% H_2O_2 for 30 min followed by 1 mol·L^{-1} HCl for an additional 30 min to disaggregate the sediment. Subsequently, 20 mL of deionized water was added and the samples were centrifuged at 4300*g* for 5 min. The supernatant was decanted and samples were dried overnight at 60 °C. Samples were subsequently incubated with 40 mL of 1% Na_2CO_3 in a shaker bath at 85 °C and 100 r/min (1 r = 2πrad). Supernatant was subsampled after 3, 4, and 5 h and analyzed for molybdate-reactive silicon using a Technicon AutoAnalyzer II according to standard methods at the Limnological Services Unit at the University of Alberta. Si concentrations at each of the three sampling periods were averaged to infer BSi concentrations at zero time.

For fossil cladocerans, 1 mL of sediment per interval was added to 15 mL 10% KOH, gently heated, and occasionally swirled for 5 h. Supernatant KOH was decanted and the sediments were diluted at least three times with distilled water. The final volume was brought to either 5 or 10 mL and subsampled to slides (50 µL per slide) using glycerine jelly stained with lignin pink as a mounting medium (Hann 1989). A phase-contrast microscope was used to identify and enumerate cladoceran remains from four slides (200 µL) or until total remains exceeded 150 per sample. Remains included postabdominal claws (daphnids), head shields, or carapaces (bosminids and chydorids). Data are reported as number of remains per milligram of dry sediment mass.

Nitrogen and carbon stable isotope ratios were determined by analyzing about 3 mg of sediment with a continuous-flow isotope ratio mass spectrometer (IRMS) using a GV Instruments IsoPrime mass spectrometer with a EuroVector

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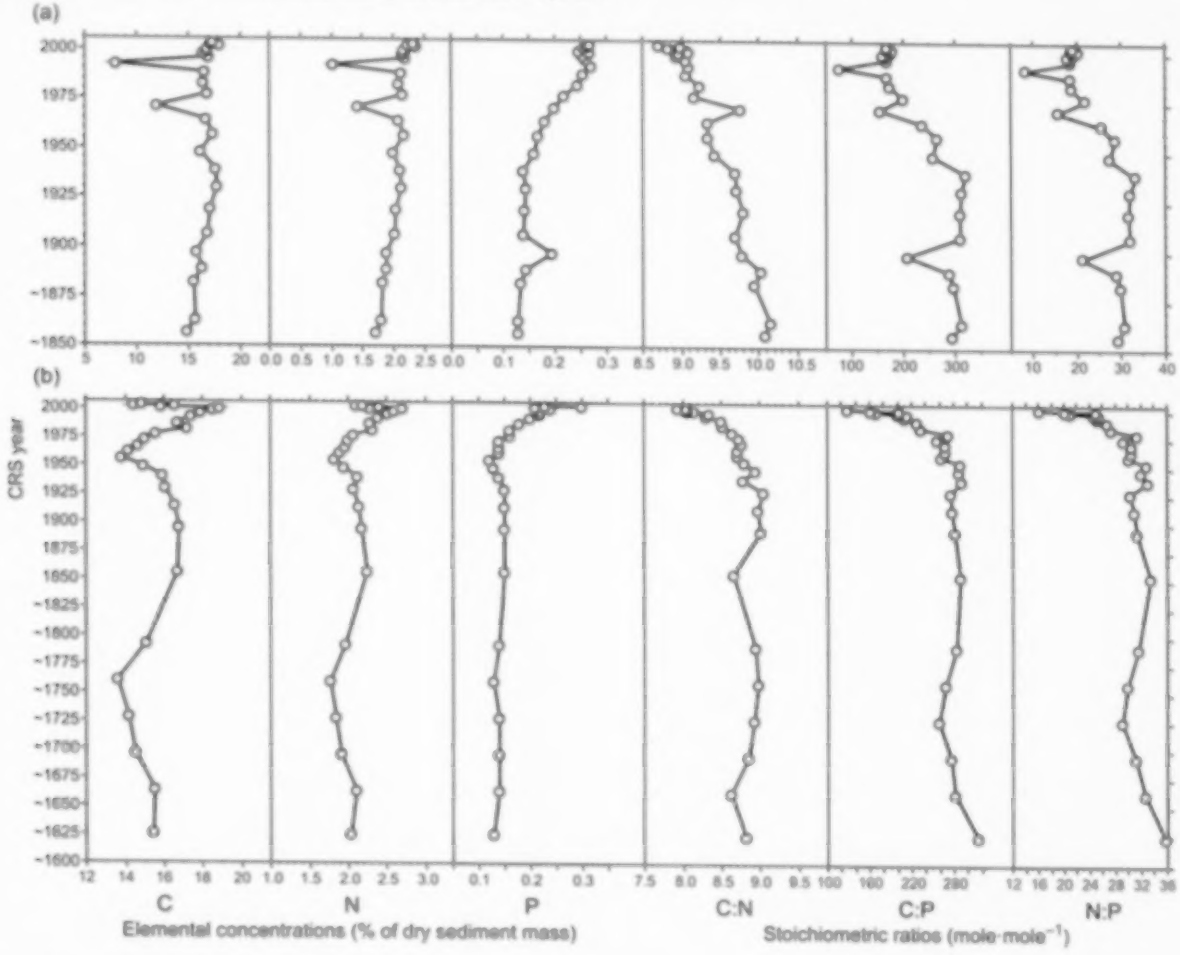
Fig. 3. Concentrations of carbon, nitrogen, and phosphorus as percent of dry mass and the molar ratios of nutrients vs. CRS dates at (*a*) site D1 and (*b*) site D3. In this and subsequent figures, dates before 1880 are extrapolated and are given only to show the relatively constant conditions before 1900. Note the varied scaling on *x* axes of the panels.



EuroEA3028-HT elemental analyzer. Analysis was performed at the Limnological Services Unit, University of Alberta. All nitrogen isotopic results are expressed in conventional δ notation relative to air as $\delta^{15}N = (R_{sample}/R_{standard} - 1) \times 1000$, where R is the ratio of measured ^{15}N to ^{14}N. A similar equation was used for calculating $\delta^{13}C$, using PeeDee dolomite as a standard.

Results

The length of the cores extended well beyond the period when reliable ^{210}Pb dates could be applied (1850 to the present). Before 1850, dates are assigned by linear extrapolation, which has the potential for considerable error. Dates are presented to show the long-term, relatively uniform deposition of nutrients and other indicators before the 20th century.

Sediment cores from both basins of the lake showed accelerated sedimentation in the latter half of the 20th century. At D1, most of the increase occurred after about 1970, increasing linearly to values in the past few years that are approximately double those in 1970 (Fig. 2). At D3, the increase began later, about 1990, but increased exponentially after that date to values two to three times higher than rates earlier in the 20th century.

Concentrations of carbon, nitrogen, and phosphorus were originally high. These and pigment and diatom analyses presented below indicate that the lake was eutrophic before European settlement. However, all nutrients increased during the latter part of the 20th century in both cores, providing evidence of increasing production and eutrophication (Fig. 3). At D1, the increase in carbon and nitrogen was slow and gradual in the early 20th century. The increase in phosphorus accelerated after about 1930. At D3, all three elements began to increase in the last few decades. The ratios of C to N, C to P, and N to P decrease in both cores in recent decades, indicating the greater increase in phosphorus than in carbon or nitrogen. The ratio of N to P, usually critical to the dominance of cyanobacteria, decreased from background values of 30:1 or greater (molar ratio) to

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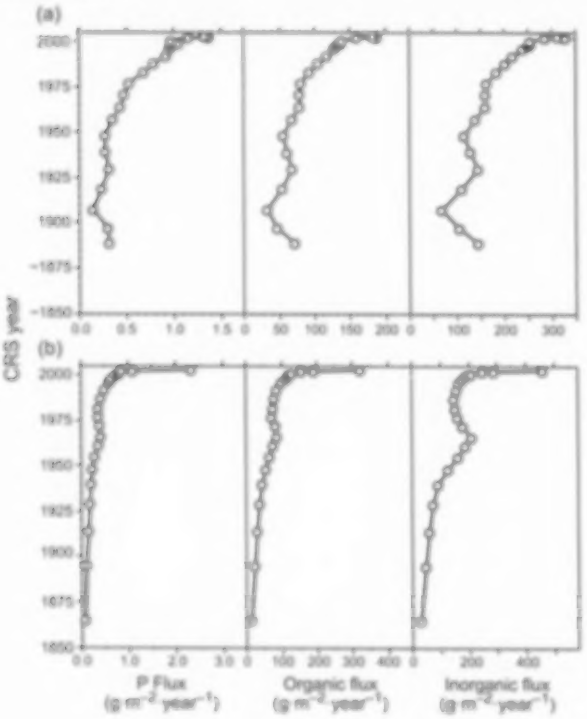

Fig. 4. Annual flux of phosphorus, organic matter, and inorganic matter to sediments ($g \cdot m^{-2} \cdot year^{-1}$) at (*a*) site D1 and (*b*) site D3 in Lac la Biche.

recent values of less than 20:1 in D1 and 16:1 in D3 (Fig. 3).

When nutrient concentrations and sedimentation rates are combined to calculate the nutrient fluxes to sediments, it is clear that annual deposition of phosphorus, organic matter, and inorganic matter have increased exponentially (Fig. 4). At D1, recent fluxes are about five times those in 1950. At D3, there was a slow increase in phosphorus flux during the period of record, which has accelerated since about 1990.

Together, these figures indicate a number of changes occurring during the 20th century. (*i*) There is an increase in soil entering the lake basin by erosion, probably bringing with it phosphorus bound to soil particles. Such erosion is very visible in areas around the lake where land uses are changing, lakeshore development is occurring, roads, pipelines, and housing sites are cleared, etc. (*ii*) After 1950, the increase in phosphorus in core D1 cannot be accounted for by erosion alone, suggesting that there are also other sources of the element. The timing of the increase suggests that sewage from the town is the most likely cause, although lakeshore development and land clearing cannot be ruled out. The increase in phosphorus at D3 occurred about 20 years later. It most likely results from cottage development, land clearing, manure from pastures, fertilizer from cultivated lands, and perhaps sewage discharge from the growing community at Plamondon (D.W. Schindler, unpublished data). Typically, phosphorus from manure, fertilizer, and sewage is much more available to algae than that from land clearing, which is usually largely bound into clay minerals. All of the sources are common problems associated with human development, and their influence has been documented at many locations in Europe and North America (Jeppesen et al. 2000; Schindler 2006).

Analyses of microbial (cyanobacteria plus algae) pigments showed that total algal abundance has increased over time in both lake basins (Fig. 5). Total chlorophyll concentrations were several-fold greater in sediments deposited since 1960, represented by sediments in the upper 20 cm of each core. However, an important caveat involving this finding is that pigments typically degrade somewhat in the upper layers of lake sediments. The stratigraphy of the chlorophyll–pheophytin ratio suggested that degradation of labile pigments was confined mainly to the uppermost 5 cm of both cores. Therefore, we remain confident that total algal abundance has increased during the latter half of the 20th century. All total chlorophyll values were comprised solely of isomers of chlorophyll *a*, which indicated a high abundance of cyanobacteria and the relative lack of other algal groups that are also capable of producing chlorophylls *b* and *c*.

Taxonomically diagnostic carotenoids showed that distinct compositional changes occurred recently in the phytoplankton community, primarily involving certain groups of cyanobacteria (Fig. 5). The presence of several diagnostic pigments (zeaxanthin, canthaxanthin, echinenone) revealed that cyanobacteria were the most common and diverse group of phytoplankton in the lake, even before European settlement. In particular, zeaxanthin levels remained relatively high and constant throughout both cores. In comparison, the canthaxanthin-inferred abundance of filamentous cyanobacteria showed a more striking increase since 1950. Recent declines in the cyanobacterial pigment echinenone in D3 suggest that certain deepwater benthic or benthic filamentous taxa have become less abundant.

Pigment profiles suggest that the abundance of other algal groups has also increased in Lac la Biche over the past few decades (Fig. 5). Diatoms have shown a gradual net increase in abundance over the past few decades as indicated by higher concentrations of diatoxanthin and fucoxanthin in the upper parts of both cores. However, rapid down-core declines in fucoxanthin levels can be partly attributed to poor preservation of this labile pigment. Higher concentrations of the cryptophycean pigment alloxanthin in the top sediment intervals of both cores further suggests that phytoflagellates have become more abundant in Lac la Biche.

Siliceous fossils also indicate that the lake was originally eutrophic, but production of silicious algae has increased in recent years (Fig. 6). The total concentration of diatom valves, chrysophyte cysts, and biogenic silica all increase in upper parts of the core.

Diatom valves are well preserved in the deeper core (D3) in contrast to the shallower core (D1), more proximal to the town site, where preservation is poor (Fig. 6). The reason for spatial heterogeneity of diatom preservation remains unclear, but it is suspected that highly alkaline pore waters have developed in the sediments deposited at D1, leading to the intensified dissolution of biogenic silica. The legacy of silica dissolution at D1 is further evidenced by much lower diatom, chrysophyte, and biogenic silica concentrations rela-

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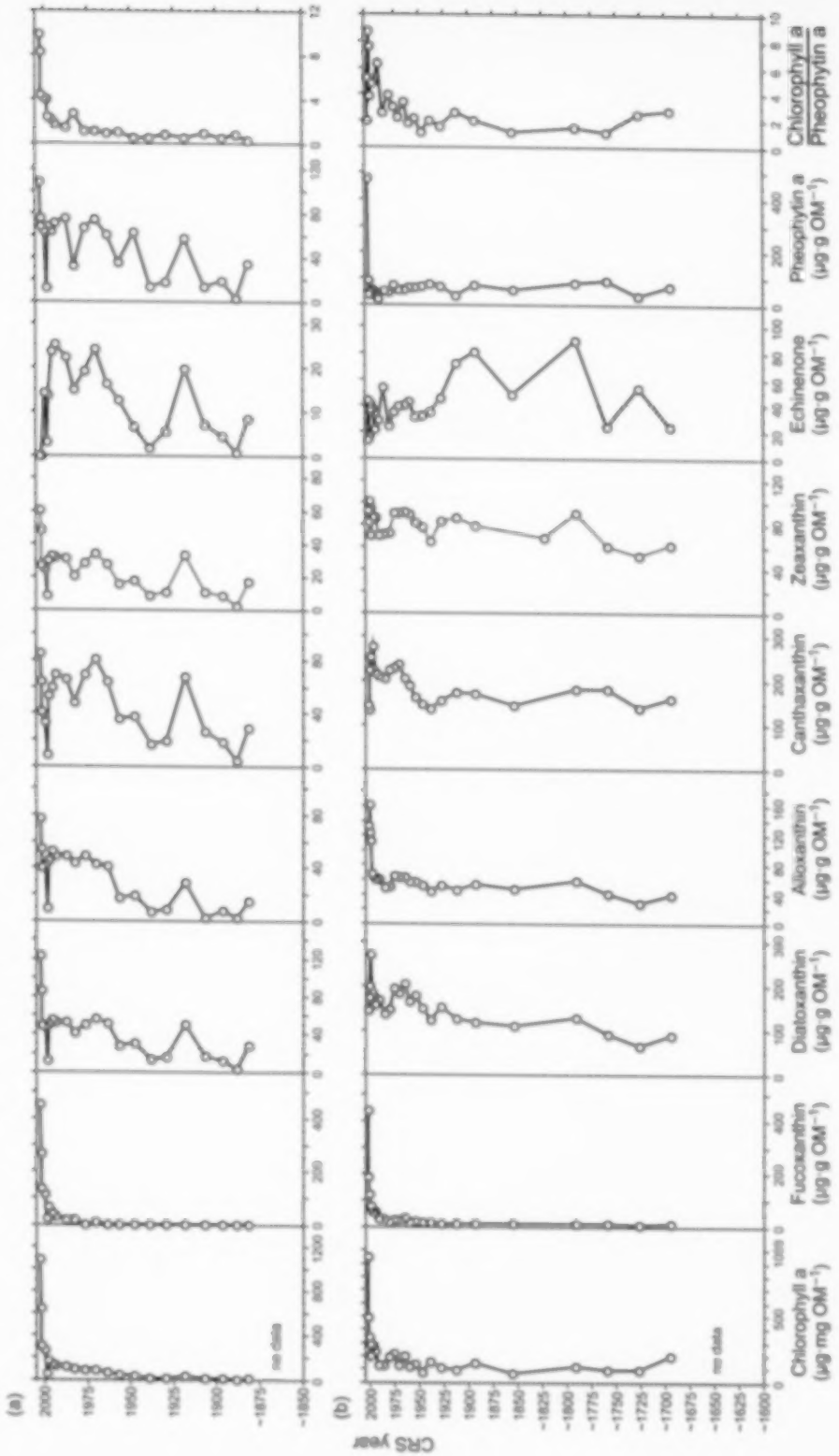

Fig. 5. Microbial (cyanobacteria and algae) pigments vs. CRS date in sediment cores from (*a*) site D1 and (*b*) site D3 in Lac la Biche.

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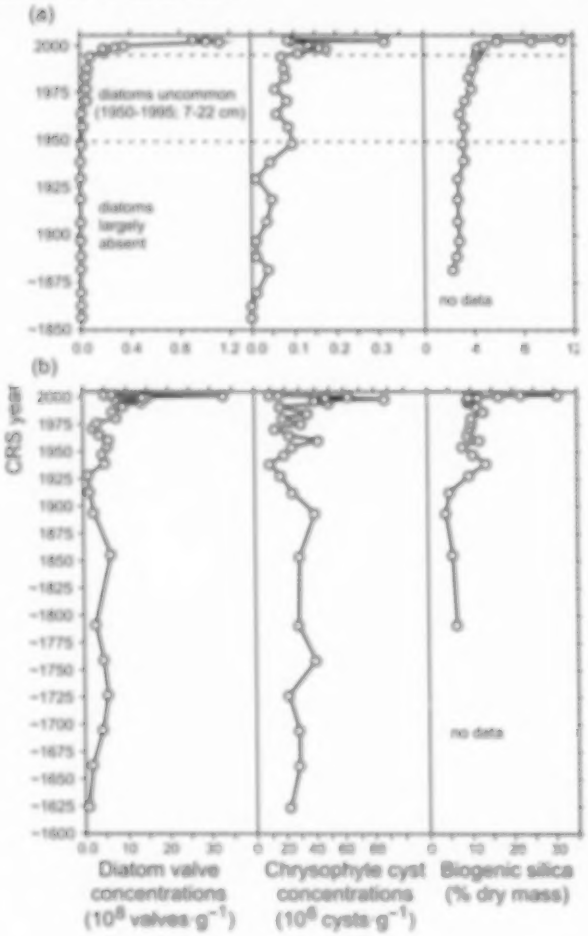

Fig. 6. The number of diatom valves and chrysophycean cysts and the concentration of biogenic silica vs. CRS dates at (*a*) site D1 and (*b*) site D3 in Lac la Biche.

tive to the core from D3 (Fig. 6). Because of the uncertainties surrounding the diatom record from the shallower site, emphasis is placed on the record from D3, which appears to capture primary features of recent ecological change.

The diatom flora from Lac la Biche is strongly dominated by mesotrophic to eutrophic planktonic taxa belonging to the genera *Aulacoseira*, *Cyclotella*, *Fragilaria*, and *Stephanodiscus*. The prevalence of planktonic diatoms is probably in part due to the D3 coring site being much deeper than the depth of the euphotic zone and several kilometres from areas that are shallow enough to support benthic photosynthesis. In core D3, benthic taxa never account for more than 15% of assemblages, and typically represent <5% (Fig. 7). Historically (i.e., pre-1850), the lake was dominated by *Cyclotella bodanica* and *Stephanodiscus minutulus*, indicating a moderately productive, but never hypereutrophic, lake regime. Beginning around 1910, diatom assemblages began to shift towards larger (*Stephanodiscus niagarae*) and more heavily silicified (*Aulacoseira granulata*) forms. This change is accompanied by increased frequencies of planktonic fragilarioid species (*Fragilaria crotonensis* and, to a lesser extent, *Fragilaria capucina*), together indicating a slight increase of lake production. Biogenic silica doubled around this time (Fig. 6). A second floristic reorganization occurred around 1990, when populations of *A. granulata* and small *Stephanodiscus* spp. decreased, displaced by *S. niagarae* and planktonic *Fragilaria* spp. This change is also reflected by several-fold increases of total diatom and chrysophyte cyst concentrations, as well as a dramatic increase in sediment biogenic silica content (Fig. 6) and the carotenoids specific to these algae (Fig. 5).

Given the available autecological data for these taxa in relation to human disturbance (Bradbury 1975; Cumming et al. 1995) and the wealth of corroborating limnological and paleolimnological data from analyses presented above and otherwise available (D.W. Schindler, unpublished data), the diatom changes expressed in Lac la Biche sediments are viewed as a first-order response to P enrichment in the absence of attendant Si limitation.

Fossil cladocerans (Crustacea) were analyzed only in the core from D3. Their remains showed rather consistent increases in fluxes of *Daphnia galeata* to sediments over time (Fig. 8). This is a small species of daphnid that is not a strong grazer, therefore usually not involved in community changes that affect algal abundance enough to cause a trophic cascade. Occasional postabdominal claws of larger *Daphnia* species were found, but there was no consistent pattern over time. *Bosmina* fossils increased from absent or very rare in the late 19th century, peaking around 1950, then decreasing slightly after that time. *Bosmina* are among the few zooplankton capable of feeding on cyanobacteria, and their increase is likely to be a consequence of the increased eutrophication of the lake. There were similar slow increases in *Chydorus* and total cladoceran remains (Fig. 8). Together, these increases roughly parallel observed increases in nutrients, diatom concentrations, and species shifts and the abundance and distribution of pigments and probably represent the overall increase in productivity of the lake.

Changes in stable isotopes with time indicate some important differences between the basins (Fig. 9). In both cores, $\delta^{13}C$ was very similar, becoming much lighter since about 1950, as expected if algal photosynthesis was responsible for an increasing proportion of carbon stored in sediments. The slight reversal of this trend since about 1975 may be due to increased inputs of allochthonous materials resulting from land clearing or lakeshore development. In the past few years, the extreme trend toward heavier carbon may be because ratios have not yet stabilized as the result of post-depositional decomposition.

In contrast, nitrogen isotope signals differed greatly between the two basins. At D1, $\delta^{15}N$ became heavier after 1950, as would be expected from the increased input of human sewage due to the construction of the wastewater treatment plant, where denitrification typically causes losses of lighter nitrogen isotopes to the atmosphere. It is noteworthy that $\delta^{15}N$ in sediments did not become lighter at the time when the sewage was diverted to Field Lake in the early 1980s, supporting our observation that nutrients from sewage still reached Lac la Biche via Red Deer Brook, the outlet of Field Lake. The strong trend toward a lighter signal in the 1990s may reflect the drought conditions in recent

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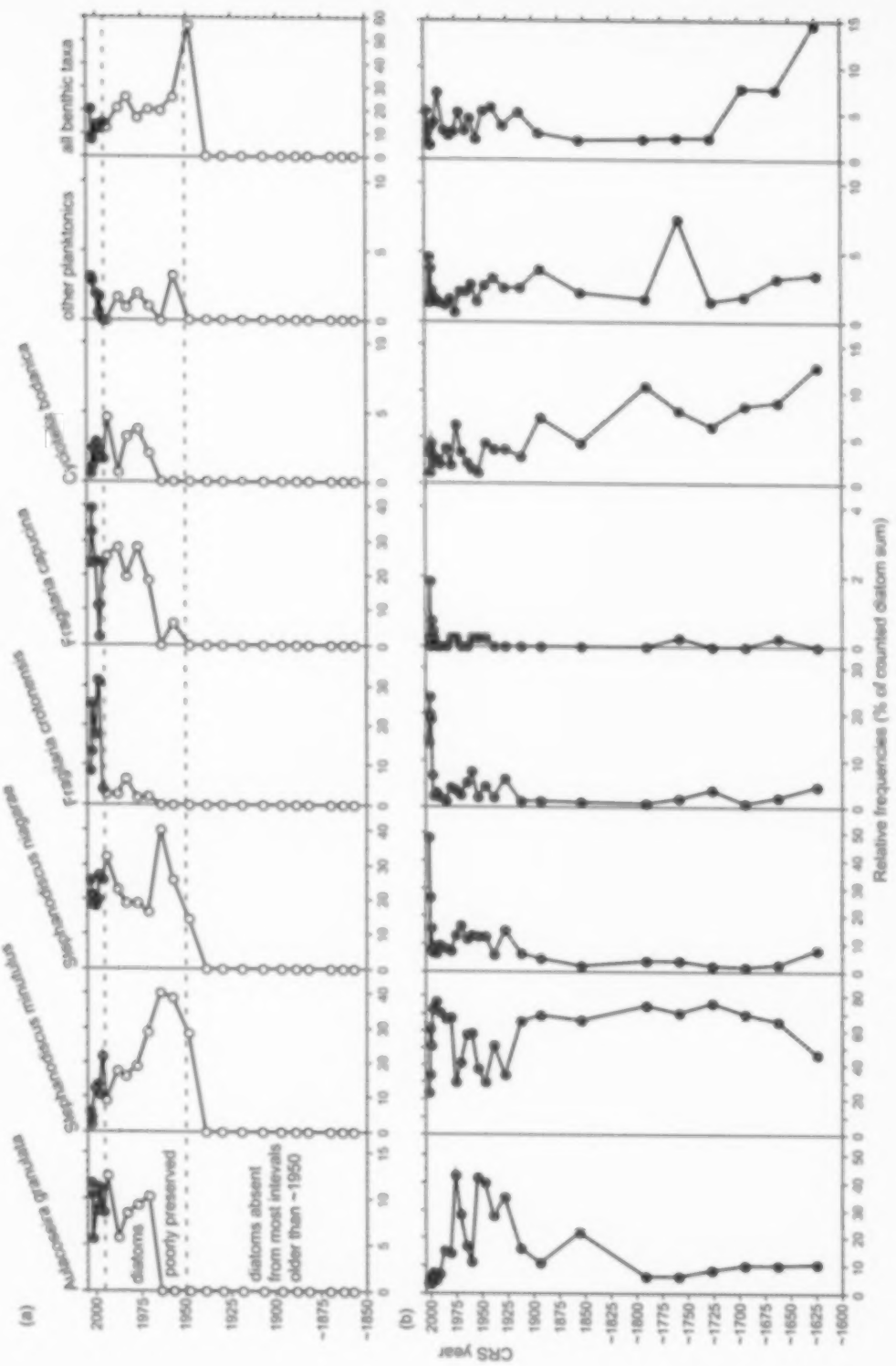

Fig. 7. Diagnostic diatom species vs. CRS dates in cores at (*a*) site D1 and (*b*) site D3 in Lac la Biche. The data points in periods of partial or total lack of preservation in core D1 are marked with open circles; those in periods with reliable preservation are marked with darker shaded circles.

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Fig. 8. Changes in fluxes of the most predominant cladoceran zooplankton in core D3.

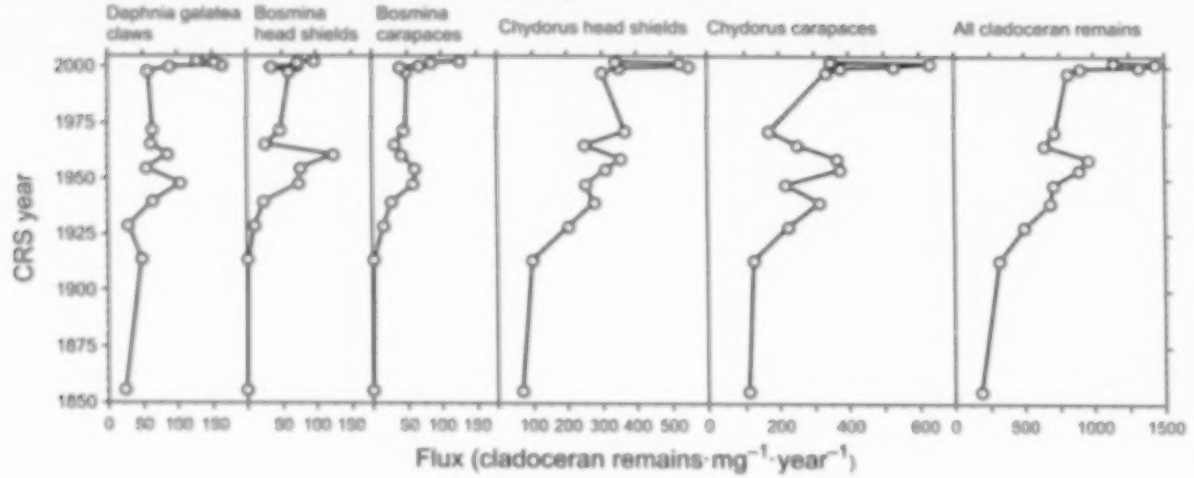
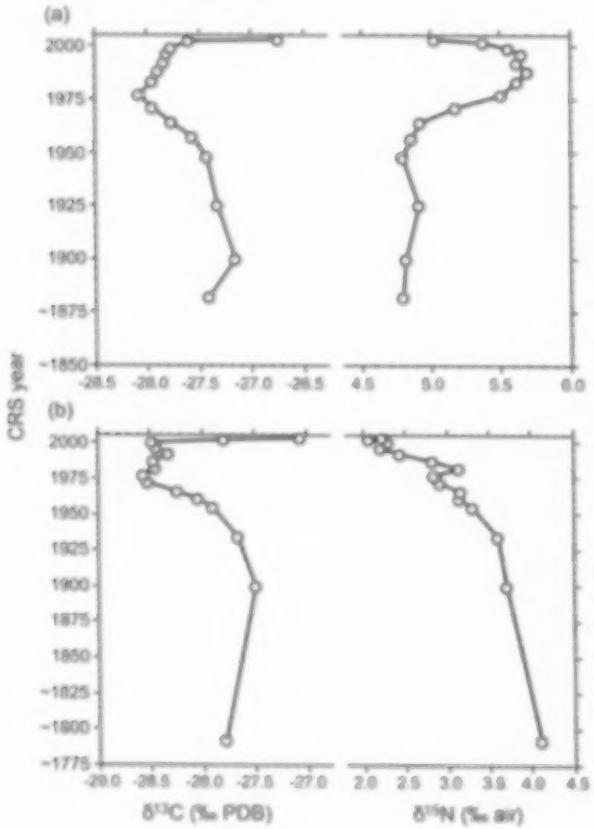


Fig. 9. $\delta^{13}C$ and $\delta^{15}N$ vs. CRS dates in sediment cores at (*a*) site D1 and (*b*) site D3 in Lac la Biche.



years, with very low flows in Red Deer Brook and other tributaries to D1, coupled with rapidly increasing abundance of nitrogen-fixing species.

In basin D3, where most human sewage is disposed of via septic tanks, there is no evidence of a heavier nitrogen signal. Instead, there is a continuing trend toward lighter nitrogen, probably reflecting the increasing importance of nitrogen-fixing cyanobacteria that residents of the area have observed.

Discussion

Taken together, our results confirm the observations of local residents that algal blooms, particularly of nuisance cyanobacteria, have increased in Lac la Biche for the past two to three decades and are intensifying. Increased accumulation rates of phosphorus and microbial pigments indicate that eutrophication accelerated in the basin nearest the community of Lac La Biche (site D1) after about 1950 and in the main basin of the lake (site D3) after 1970. Eutrophication was also demonstrated by increases in diatom numbers and in biogenic silica deposition and by a shift toward diatom species indicative of more productive conditions.

Increased annual fluxes of inorganic matter indicate that erosion in the catchment of the lake has also increased. Local soils contain apatite, and erosion is partly responsible for the nutrient increases in sediments, especially early in the 20th century. Such changes are commonly observed when forested land is changed to agricultural or urban uses, when roads are built, and when cottage development causes erosion near the lakeshore. All of these happened in the Lac la Biche catchment. After the mid-20th century, increased concentrations of phosphorus and nitrogen indicate that human sewage and (or) animal manure are likely additional nutrient sources. The N–P ratios of sewage and manure are typically <4:1 by weight, causing the decline in N–P ratios in sedimented material in recent years. The declining N–P ratio over time indicates that nutrient conditions have become increasingly favourable for nitrogen-fixing cyanobacteria, species that are commonly associated with nuisance blooms of algae.

Biogenic silica does not decline to near zero before 1950 in core D1, despite the poor preservation of diatoms. We believe that the high background of biogenic silica is the result

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of the decay of microfossils such as sponge spicules, phytoliths, and gemmoscleres in D1. Also, chrysophycean stomatocysts and siliceous protozoan plates were still abundant in these older sediments. Also, although identifications were not possible, some diatom fragments were still found at the base of the core. It should also be recognized that the alkali treatment in the analysis for biogenic silica represents a compromise between complete dissolution of biogenic compounds and the beginning of an attack and solubilization of clay minerals. Inorganic phytosilicates may also be dissolved by the technique. In short, we expect that the method would show a background above zero even when recognizable diatom frustules are not present.

Preliminary evidence from the fossil Cladocera suggests that disrupted trophic relationships as the result of declining populations of walleye (*Sander vitreus*) and other predators has probably not played a large role in the eutrophication of the lake. If such a "trophic cascade" had been initiated by the heavy fishing pressure that is known to have occurred, *Daphnia* numbers should have declined during the 20th century. Instead, there is a slow increase in the flux of *Daphnia* remains, which is probably the result of a general increase in phytoplankton during the period. In view of the documented human travel through the area, the appearance of *Bosmina* in the early 20th century may have been an accidental introduction from other lakes. Both *Bosmina* and *Chydorus* also increased slowly over time. The most plausible explanation for the observed pattern is that all cladoceran taxa responded to the increasing productivity of the lake.

The constant rates of deposition of sediments and of chemical and biological proxies in the period before 1900 indicate that it is unlikely that the small aboriginal population and the early period of European settlement were responsible for eutrophication of Lac la Biche. Most proxies began to change in consistent ways early in the 20th century, during the period when land clearing in the basin began, the result of increased influx of European settlers. All proxies indicate that eutrophication accelerated greatly in the latter part of the 20th century, when land clearing increased, summer cottage development accelerated, and waterborne disposal of sewage began, as described in the Introduction.

It is not a new discovery that many lakes of the prairie provinces were naturally eutrophic. Hickman and Klarer (1981) and Forbes and Hickman (1981) had reached similar conclusions for Lake Isle and Lac Ste. Anne, and Hickman et al. (1984) had reached similar conclusions for Lake Wabamun. Similarly, records of preserved sedimentary microbial pigments from three closed-basin lakes located within the Palliser Triangle (Vinebrooke et al. 1998) and four lakes from the Qu'Appelle Valley (Dixit et al. 2000) revealed high inherent levels of production extending back several centuries.

However, there is accumulating paleoecological evidence that eutrophication of prairie lakes has accelerated since the mid-20th century. Blais et al. (2000) found that Lake Isle and Lac Ste. Anne had undergone recent increases in eutrophication. Similarly, Hall et al. (1999) and Dixit et al. (2000) showed that resource use (cropland area, livestock biomass) and urbanization (nutrients in sewage) were the key drivers of recent increases in algal biomass in lakes of the Qu'Appelle Valley drainage system. Intensification of agriculture and urbanization of the catchment surrounding Lac Saint-Augustin during the 20th century also resulted in eutrophication-induced shifts in diatoms and major increases in sedimentary pigment and nutrient concentrations (Pienitz et al. 2006). Neufeld (2005) documented that phosphorus inputs per unit area of catchment to Lac la Biche from pastures and cropland were approximately double the areal inputs from forested catchments in the area. Moser et al. (2002) and Hazewinkel (2006) present evidence that airborne nutrient sources may also contribute to the eutrophication of prairie lakes. Thus, both our study and several others document that nutrient inputs to lakes have increased greatly throughout the western prairies in the past few decades. Similar results were reported for Lake Winnipeg (Kling 1998). In addition to increased nutrient inputs, Schindler (2006) notes that increased water residence times of lakes resulting from recent warming and drought are likely to contribute to eutrophication by causing increased retention of nutrients in lakes.

Recent eutrophication, even in naturally eutrophic lakes, also appears to be common and widespread in other parts of the world. For example, Rasanen et al. (2006) report the further eutrophication of naturally eutrophic lakes in Finland. Umbanhowar et al. (2003) report recent hypereutrophication of Lake Volney, Minnesota, USA, resulting from livestock culture in the catchment. Reavie et al. (2000) report that of 11 naturally eutrophic lakes in British Columbia, Canada, seven had become more eutrophic as the result of recent human activity. Together, these results suggest that declining water quality accompanying human activity is widespread, despite our advances in knowledge of preventing and reversing eutrophication. When reliable long-term monitoring is not available, multiproxy paleolimnological studies can be used to deduce lake history from the sedimentary record, as we have shown here.

Climate change since 1970 has reduced lake levels and outflow volumes, increased summer surface temperatures, and increased the salinity of lakes in the Canadian prairies (Vinebrooke et al. 1998; Schindler et al. 2004). In some cases, the result has been the proliferation of filamentous benthic cyanobacteria and green algae, even without increases in phosphorus loading. Therefore, climate warming has the potential to synergistically amplify the impacts of human eutrophication on prairie lakes. Such lakes are likely to resist recovery efforts, with internal phosphorus loading persisting for years after external sources have been controlled. Similar conditions have been observed previously (Sondergaard et al. 2001; Schindler 2006).

All of our proxy indicators of eutrophication indicate similar timing for dates of onset and rates, lending credence to the claims of local residents about the condition of the lake. That studies based on a few proxies can result in misinterpretations is well known. For example, Warwick (1980) initially found that interpretation of chironomid remains led to the wrong conclusions about eutrophication in the Bay of Quinte, Ontario. His subsequent inclusions of additional proxies showed that the response of chironomids was confounded by land clearing, which caused massive erosion of silt and clay into the bay, which in turn diluted the increased sedimentation of organic matter resulting from eutrophica-

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tion. Rosén et al. (2001, 2003) found that when several proxies of climate warming were compared, there were inconsistencies between the reconstructions provided by each. They attributed these to the lack of adequate modern analogues for some proxies and the effects of long-distance transport of pollen to their study lakes. They concluded that multiproxy studies leading to a consensus reconstruction are necessary to obtain reliable indications of past conditions in lakes. We agree with their conclusion.

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Validation of annual growth rings in freshwater mussel shells using cross dating

Andrew L. Rypel, Wendell R. Haag, and Robert H. Findlay

Abstract: We examined the usefulness of dendrochronological cross-dating methods for studying long-term, interannual growth patterns in freshwater mussels, including validation of annual shell ring formation. Using 13 species from three rivers, we measured increment widths between putative annual rings on shell thin sections and then removed age-related variation by standardizing measurement time series using cubic splines. Initially, cross dating was a valuable quality control technique allowing us to correct interpretive and measurement errors in 16% of specimens. For all species, growth varied among years but was highly synchronous among individuals. Standardized measurement time series of 94% of individuals were significantly correlated with species master chronologies, and mean interseries correlations ranged from 0.37 to 0.96. Growth was also synchronous among species, even from different rivers, and growth was negatively correlated with mean annual streamflow for most species except *Quadrula pustulosa* from a regulated dam tailrace. Highly synchronous growth and the strong relationship to streamflow showed that large-scale environmental signals generated non-age-related variation in mussel growth giving strong support for annual formation of the growth increments we measured. Cross dating can be a valuable technique for studying freshwater mussel growth providing quality control, validation of annual rings, and reconstruction of long-term growth histories.

Résumé : Nous examinons l'utilité des méthodes dendrochronologiques de datation croisée dans l'étude des patrons interannuels de croissance à long terme chez les moules d'eau douce et, en particulier, pour la validation de la formation des anneaux annuels sur la coquille. Chez 13 espèces provenant de trois rivières, nous avons mesuré la largeur des incréments entre les anneaux annuels présumés sur des coupes minces de coquille, puis nous avons éliminé la variation due à l'âge en standardisant les séries chronologiques de mesures à l'aide de fonctions splines de degré 3. Au départ, la datation croisée s'est avérée être une technique précieuse de contrôle de la qualité qui nous a permis de corriger les erreurs d'interprétation et de mesure chez 16 % des spécimens. Chez toutes les espèces, la croissance varie d'une année à l'autre, mais est très synchronisée entre les individus. Les séries chronologiques standardisées des mesures de 94 % des individus sont en corrélation significative avec les chronologies principales des espèces et les corrélations moyennes entre les séries varient de 0,37 à 0,96. La croissance est aussi synchronisée entre les espèces, même de rivières différentes, et la croissance est en corrélation négative avec le débit moyen annuel pour la plupart des espèces, à l'exception de *Quadrula pustulosa* dans le canal de fuite d'un barrage de régulation des eaux. La croissance fortement synchronisée et la corrélation élevée avec le débit montrent que des signaux environnementaux à grande échelle causent une variation non reliée à l'âge dans la croissance des moules, ce qui confirme nettement la formation annuelle des incréments de croissance que nous avons mesurés. La datation croisée peut être une méthode précieuse pour étudier la croissance des moules d'eau douce en fournissant un contrôle de la qualité, en validant les anneaux annuels et en permettant la reconstruction du déroulement de la croissance sur une grande échelle temporelle.

[Traduit par la Rédaction]

Introduction

Annual growth increments are often prominent features of bivalve shells and can provide a wealth of information about age and growth of these animals. Validation of the annual formation of growth increments is an essential prerequisite for deriving age and growth information from shell annual rings or analogous structures in any organism (Beamish and McFarlane 1983). In addition to confirming timing of growth increment formation, validation studies decrease errors in interpretation of these features (DeVries and Frie 1996; Campana 2001; Haag and Commens-Carson 2008). Although annual formation of growth increments has been demonstrated for many marine bivalves, few validation studies have been conducted for freshwater mussels (see Haag and Commens-Carson 2008). To utilize the full potential of growth increments as indicators of mussel age and growth, annual formation of shell rings needs to be validated for a large number of species across different habitats and regions using robust, repeatable methods.

Most validation studies for freshwater mussels have used mark–recapture methods involving marking shells, returning marked mussels to their habitat, and then retrieving them at least one year later to examine shell growth deposited after

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marking (e.g., Neves and Moyer 1988; Howard and Cuffey 2006; Haag and Commens-Carson 2008). This approach can be effective but is time consuming (requiring at least one full year) and often suffers from a low return of marked animals in dynamic stream habitats. Further, handling of study specimens causes deposition of disturbance rings that are superficially similar to annual rings and can result in reduced growth, both potentially leading to erroneous conclusions about the periodicity of growth rings (Haag and Commens-Carson 2008). The disadvantages of mark–recapture techniques for freshwater mussels highlight a need for alternative and less intrusive methods of independently validating putative shell annual rings.

Cross dating is a fundamental technique of dendrochronology by which high frequency patterns of annual variation in tree ring width (reflecting variation in growth) are used to validate the timing of ring formation and to ensure that all increments have been correctly identified and assigned the correct calendar year (Douglas 1939). Because growth increments in trees are influenced by climate, long-term growth chronologies of individual trees can be highly synchronous within a population and the mean chronology of a population (referred to as the master chronology) is often correlated with annual variations in climate. Therefore, interpretation of annual ring formation for an individual tree can be validated if the standardized measurement time series for that individual is correlated with the master chronology for the population; this is referred to as the sample–master correlation (Grissino-Mayer 2001; Esper et al. 2002). Further, aligning and cross matching each standardized measurement time series with the master chronology can reveal growth anomalies or errors in ring interpretation and can allow archeological dating of wood specimens of unknown age provided a master chronology for that species already exists (Douglas 1921; Alestalo 1971; Grissino-Mayer 2001).

Dendrochronological techniques have been applied to other taxa such as freshwater and marine fishes, corals, and marine mollusks forming the basis of an emerging science known as "sclerochronology" (Pereira et al. 1995; Strom et al. 2004; Helama et al. 2006). Because growth of poikilothermic aquatic animals is also directly influenced by climate, as well as by hydrologic conditions, sclerochronological cross dating has been applied to validate and study annual growth increments in fish otoliths and fin rays, marine bivalve shells, and shells of the Holarctic pearl mussel *Margaritifera margaritifera* (Pereira et al. 1995; Black et al. 2005; Helama et al. 2006).

We apply cross dating to evaluate the assumption of annual deposition of shell rings in 13 freshwater mussel species from three rivers in the southeastern United States. First, we examine patterns of shell ring formation and assess whether observed levels of variation are useful for cross dating. Second, we examine synchronicity of growth among individuals in a population and use the resulting interseries correlations to validate annual rings and identify growth anomalies or potential errors in interpretation of shell thin sections. Third, we compare master chronologies of different species within and among rivers to explore the generality of observed patterns of variation. Fourth, we examine relationships between annual variation in mussel growth and climate and hydrological variables. Finally, we discuss how cross dating can be used either with mark–recapture methods or as a stand-alone method for validating the assumption of annual formation of shell rings in freshwater mussels.

Materials and methods

Study sites and species

Mussels were collected from one site in the Little Tallahatchie River, Panola County, Mississippi (34°23′56″N, 89°47′33″W), one site in the St. Francis River, Cross County, Arkansas (35°16′12″N, 90°34′58″W), and at two sites in the Sipsey River, Pickens–Greene Counties, Alabama (33°07′16″N, 87°55′08″W; 33°05′16″N, 87°57′27″W). All four sites support diverse and abundant mussel communities, but physical habitat conditions differ greatly among the streams. The Little Tallahatchie River is regulated and impounded; our site was located in the dam tailrace below a major storage reservoir (Sardis Reservoir) and is impounded by a low-head dam about 2–3 km downstream of the site (Haag and Warren 2007). Substrate at the Little Tallahatchie River site was composed primarily of sand, and water depth was approximately 3.0 m. The St. Francis River is largely unregulated in the vicinity of our study site, but much of the watershed is affected by channelization and water diversion projects; land use in the watershed is dominated by large-scale, intensive agriculture and the river receives heavy runoff from these activities (Ahlstedt and Jenkinson 1991). Substrate at the St. Francis River site was mostly silt with some sand, and water depth was 1.0–1.5 m. The Sipsey River is unregulated and unmodified and the watershed is mostly forested; consequently, water quality is high and the river supports one of the most intact aquatic communities in the region (McCullagh et al. 2002). The Sipsey River sites were within 10 km of each other and had stable gravel and sand substrates and water depths from ~0.1 to 1.2 m. At all sites, we collected mussels from within about 100–200 m segments of stream channel.

Thirteen mussel species were used in this study (Table 1; total shells examined = 203), and with the exception of *Elliptio crassidens* and *Fusconaia ebena*, all 13 study species were common constituents of mussel communities at the study sites (Table 1). We included *E. crassidens* and *F. ebena* because the high number of rings present in our specimens allowed construction of lengthy growth chronologies. Study species represented a diversity of life history traits, including host fish use (Haag and Warren 2003), fecundity (Haag and Staton 2003), and longevity. Most study species grow relatively slowly and live to >30 years. However, *Lampsilis ornata*, *Lampsilis teres*, and *Potamilus purpuratus* grow rapidly and rarely exceed 20 years of age (W.R. Haag, unpublished data). Growth and longevity of *Quadrula quadrula* and *Quadrula verrucosa* are intermediate; both of these species commonly live >20 years, but we had no older specimens in this study. The assumption of annual ring formation has been validated previously at these same study sites using mark–recapture methods for all species in the present study except *E. crassidens*, *F. ebena*, *Obovaria unicolor*, and *Pleurobema decisum* (Haag and Commens-Carson 2008).

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Table 1. Cross-dating statistics for 13 mussel species from southeastern USA.

Species	Time series	*N*	Optimal spline flexibility	% of individuals validated		Mean interseries *R* (Pearson's)	
				Before QC	After QC	Before QC	After QC
Sipsey River							
Elliptio arca	1974–2005	21	24	76	95	0.39	0.50
Elliptio crassidens	1949–1999	10	40	100	100	0.50	0.50
Fusconaia cerina	1954–2005	29	24	76	93	0.25	0.49
Fusconaia ebena	1955–1999	5	16	80	80	0.37	0.37
Lampsilis ornata	1982–2005	23	24	87	91	0.48	0.60
Obovaria unicolor	1963–1998	8	8	88	88	0.37	0.55
Pleurobema decisum	1955–1999	12	16	50	92	0.20	0.37
Quadrula asperata	1966–2005	26	32	77	96	0.46	0.49
Quadrula (Tritogonia) verrucosa	1982–1999	17	32	82	100	0.42	0.56
St. Francis River							
Lampsilis teres	1993–2002	9	16	67	100	0.44	0.76
Potamilus purpuratus	1995–2003	8	8	75	100	0.44	0.96
Quadrula quadrula	1991–2003	5	24	80	100	0.36	0.49
Little Tallahatchie River							
Quadrula pustulosa	1966–2003	17	32	65	77	0.38	0.52

Note: *N*, number of shells examined for each population; optimal spline flexibility, the value that resulted in the highest mean interseries correlation for that species; % validated, the percentage of individuals with measurement time series that were significantly and positively correlated with the master chronology for that species; QC, quality control.

Collection and preparation of shells

We collected live mussels, when possible, for all species except *P. decisum* by snorkeling and using SCUBA from 1996 to 2006. *Pleurobema decisum* is listed as endangered under the US Endangered Species Act and no live individuals were collected; we collected only freshly dead shells. In addition, we used freshly dead shells of other species if we were unable to collect sufficient numbers of live animals.

In the laboratory, we prepared radial thin sections (~300 μm thick) from one valve of each specimen using a low-speed saw with a diamond-impregnated blade (Buehler Ltd., Lake Bluff, Illinois). Our thin-section preparation methods are described in detail in Haag and Commens-Carson (2008) and are based on standard methods for bivalves (e.g., Clark 1980; Neves and Moyer 1988; Veinott and Cornett 1996).

Interpretation and measurement of shell rings

All thin sections were interpreted independently by two experienced observers using a binocular microscope. We identified annual rings and differentiated them from non-annual shell rings (e.g., disturbance rings) following criteria described by Haag and Commens-Carson (2008). Briefly, annual rings were continuous from the shell exterior to the umbonal region and were not associated with a disruption of the continuity of the prismatic shell layer as seen frequently in disturbance rings. In many cases, annual rings curved abruptly within the prismatic layer and were surrounded by a clear halo within this region of the shell. If the two observers differed in their interpretation of an increment and could not reach consensus following re-examination of the specimen, the specimen was excluded from further analysis.

We measured annual growth increments using a binocular microscope and digital camera interfaced with video-imaging software. For each specimen, we initiated measurements at the most recent complete growth increment (i.e., the year prior to collection) and worked backwards in time, allowing us to associate a specific calendar year with each growth increment. We measured growth increments as the linear dorsoventral distance (to the nearest 0.1 mm) between adjacent annual rings at the interface between the prismatic and nacreous shell layers (Fig. 1*a*); we used this measurement point because it offered a precise and unambiguous landmark that was usually unaffected by erosion of the periostracum or other external shell damage. Increments measured linearly may slightly underestimate growth in some individuals relative to curvilinear measurements made along the shell surface but the degree of curvature between increments is usually very small and the difference between these two measurements is negligible. We measured growth increments of three individuals of *F. ebena*, *Quadrula asperata*, and *L. ornata* (representing species of slow, intermediate, and fast growth, respectively), using both linear and curvilinear techniques. Correlation coefficients between the resulting standardized growth indices (see ARSTAN methods below) for the two measurement techniques were ≥0.95 for all species. Shell erosion in the umbonal region of many specimens precluded creation of a complete growth series, but in general, growth increments were incomplete or absent for no more than five of a specimen's earliest years.

Cross dating and analysis of shell ring patterns

We cross-dated each standardized measurement time series within a species using the dendrochronology program, COFECHA (http://web.utk.edu/~grissino/software.htm; Holmes 1983; Grissino-Mayer 2001). We generated standardized index series by removing age-related variation in growth using a flexible cubic spline (Cook and Peters

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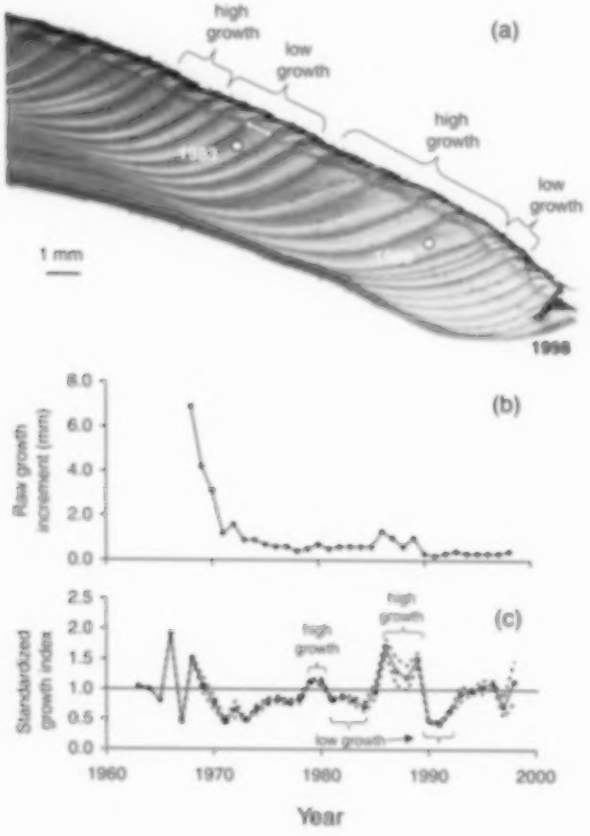
Fig. 1. Example of master chronologies for *Obovaria unicolor* from the Sipsey River, Alabama. (*a*) Thin section of specimen showing annual variation in growth increment width including alternating periods of high and low growth. From left to right, periods represent 2, 4, 4, and 3 years of growth, respectively. Years are indicated on the cross section for reference purposes. White arrow in 1983 shows measurement dimension for growth increments. (*b*) Raw measurement time series for specimen figured above showing strong age-related variation in growth. (*c*) Master chronology for *Obovaria unicolor* ($n = 8$ individuals) showing annual variation in growth unrelated to age. *y* axis is a unitless index of growth with mean = 1. Indicated high and low growth periods correspond to those in panel *a*. Standard errors for each year are indicated by broken lines.



1981). For each species, we determined optimal spline flexibility by applying a range of flexibilities and choosing the value that resulted in the highest mean interseries correlation (R) within the master chronology (Black et al. 2005; Helama et al. 2006; see below and Table 1). For each species, we generated a master growth chronology by computing the average standardized growth increment for each calendar year of record. We then correlated standardized measurement time series with an adjusted master chronology that was created by omitting the series of that particular individual (Grissino-Mayer 2001). Individuals that were significantly ($\alpha < 0.05$) and positively correlated with the master chronology were considered validated and the mean interseries correlation coefficient across all individuals was used to assess the overall robustness of the annual growth pattern for each species.

We examined each specimen for potential interpretive errors by lagging each standardized measurement time series forward and backward by 1–3 years and correlating the lagged series with the master chronology (Grissino-Mayer 2001). If the correlation coefficient was strongest with the series in its original placement in time, we considered that the shell thin section had been interpreted correctly (Holmes 1983). If correlation coefficients were stronger after lagging, we re-examined the specimen for potential errors in interpretation. If interpretive errors were found after re-examination (e.g., missed annual rings, nonannual rings counted as annual), we remeasured increment widths for the entire specimen and constructed a corrected standardized measurement time series (Black et al. 2005). If no obvious interpretative errors were found, we retained the specimen in the data set with its original measurements. After this quality control technique was completed, we repeated the entire cross-dating procedure using the corrected data set.

To illustrate this cross-dating quality control procedure, we simulated an error for one *Fusconaia cerina* individual by combining the 1979 and 1980 growth increments for this shell, thus displacing the series by –1 years. This dropped the individual's correlation coefficient with the adjusted master chronology from 0.54 to 0.15. When this individual was run through the COFECHA cross-dating routine, the program accurately detected this error and suggested that by lagging the series by –1 years the correlation coefficient could be improved to 0.42.

Following cross dating and quality control, we transformed corrected, raw growth increment data into growth indices using another dendrochronology software package, ARSTAN (http://www.ldeo.columbia.edu/res/fac/trl/public/publicSoftware.html; Cook and Holmes 1984; for a discussion of ecological applications of ARSTAN and COFECHA, see Drake and Naiman 2007). Similar to COFECHA, ARSTAN removes age-related growth trends by using a detrending function, but users specify one of a number of detrending algorithmic options. To retain as much of the low-frequency climatic signature as possible, "stiffer" (e.g., negative linear and negative exponential) functions are often used at this stage (Fritts 1966). We therefore standardized each individual using a simple negative exponential curve and divided the actual increment value by that predicted from the curve. Detrending and removal of autocorrelation resulted in a unitless, standardized index of growth for each year with an average of 1; values above 1 represent above-average growth and values below 1 represent below-average growth. Using these growth indices we cross-correlated master growth chronologies of all species to examine similarities in growth patterns among species. For each species, we correlated the annual mean growth index with mean annual air temperature and mean annual streamflow (Pearson's product moment analysis; JMP statistical software, version 5.1, SAS Institute, Cary, North Carolina). We used data from the nearest available weather station or streamflow gage as follows: Little Tallahatchie River, Sardis Dam weather station, 1949–2004, Sardis Dam discharge data, 1947–2005 (US Army Corps of Engineers, Sardis Lake

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Field Office, Sardis, Mississippi); St. Francis River, Memphis International Airport weather station, 1940–2006, US Geological Survey streamflow gage 07047800 at Parkin, Arkansas, 1931–2006; Sipsey River, Tuscaloosa County Airport weather station, 1949–2006, US Geological Survey streamflow gage 02446500 at Elrod, Alabama, 1929–2006.

Results

Master growth chronologies for each species ranged from 9 to 52 years (Table 1). Alternating periods of high and low growth were clearly visible on thin sections (Fig. 1*a*), and these patterns were borne out in the master growth chronologies (Figs. 1*b* and 1*c*). In addition, pointer years of exceptionally high or low growth were evident in many standardized measurement time series and were often similar among species (Fig. 2, note years 1965–1967, 1968, and 1987). The variation around the standardized growth index (mean = 1) was of similar magnitude for all species (coefficients of variation = 19.7%–39.4%).

Cross dating identified 49 specimens as potentially having interpretive or measurement errors. After re-examination of these specimens, we identified and corrected errors in 33 specimens (16% of total specimens examined in study). Three individuals were discarded because of extensive shell erosion that prevented a conclusive reassessment of rings, and we retained unchanged the measurement time series for 13 individuals for which we could identify no errors. Corrected errors were a result of both faulty interpretation of thin sections (e.g., inaccurate identification of annual shell rings) and errors in measurement of growth increments. Errors in the number of annual rings identified for an individual ranged from –5 (e.g., five nonannual rings initially identified as annual) to +2 (e.g., two annual rings missed). However, most errors involved a discrepancy of only one or two rings (the mean of absolute values of all corrected discrepancies = 1.6 rings). Correcting these errors increased the number of individuals validated and the strength of interseries correlations for most species (Table 1; see below).

Long-term patterns of mussel growth were highly synchronous among individuals within all species. After correcting for errors identified by cross dating, 94% of total individuals examined were considered validated (standardized measurement time series significantly correlated with the species' master chronology; Table 1). Greater than 90% of individuals were validated for all species except *O. unicolor* (87% validated) and *Quadrula pustulosa* (77% validated). Mean interseries correlations after quality control ranged from 0.37 to 0.96, showing that most individuals in a population share a high percentage of non-age-related variation in growth. The three fastest-growing species had the highest mean interseries correlations: *L. ornata*, *L. teres*, and *P. purpuratus* (0.60–0.96). Otherwise, interseries correlations for the majority of species were similar and were near 0.50 (average across all species = 0.55; Table 1).

Growth was also similar among many species (Table 2). At the Sipsey River, master growth chronologies of all species were significantly and positively correlated with at least two other species in the assemblage. *Elliptio crassidens*, *F. cerina*, and *F. ebena* were each correlated with all but one of the other species and *E. arca* and *Q. asperata* were correlated with all but two species. Master growth chronologies of all species in the St. Francis River were strongly and positively correlated with each other. Growth of many species was also similar among the Sipsey and St. Francis rivers. Growth of *Q. pustulosa* in the tailrace of Sardis Dam on the Little Tallahatchie River was correlated with only one species (*E. arca*, Sipsey River) but the correlation coefficient indicated a negative relationship; this was the only significant negative correlation among any pair of species.

Growth of nearly all species was significantly negatively correlated with mean annual streamflow (Table 2). Growth of *P. decisum* and *Q. verrucosa* was also negatively correlated with streamflow but these correlations were not significant ($P = 0.19$ and 0.11, respectively). *Quadrula pustulosa*, collected from the tailrace of Sardis Reservoir, was the only species that did not show a relationship between streamflow and growth. No significant correlations between annual growth and annual air temperature were detected.

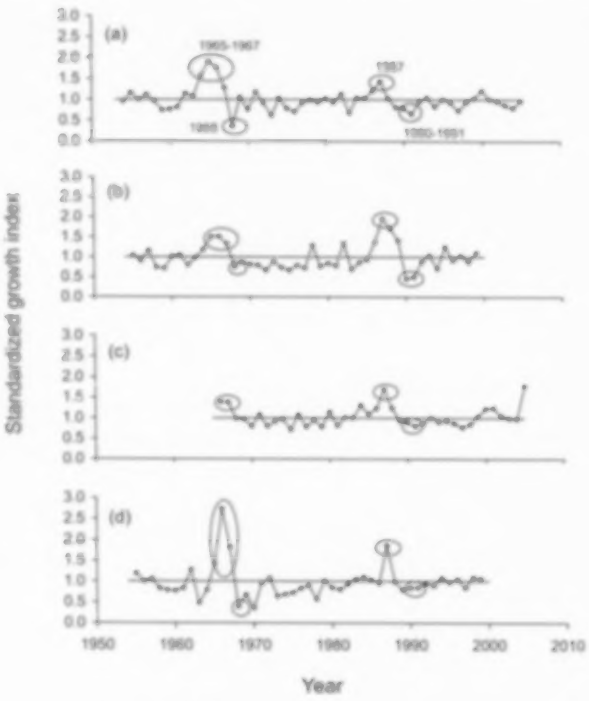

Fig. 2. Non-age-related variation in annual growth of four species of from the Sipsey River, Alabama: (*a*) *Fusconaia cerina* ($n = 29$); (*b*) *Fusconaia ebena* ($n = 5$); (*c*) *Quadrula asperata* ($n = 26$); and (*d*) *Pleurobema decisum* ($n = 12$). Data are standardized master chronologies for each species. Ellipses (identified in *a*) highlight pointer years of exceptionally high or low growth that are shared by at least three species. Horizontal lines represent average annual growth for the population (growth index = 1). Standard errors were similar to those found in Fig. 1 and are excluded for graph clarity.

Discussion

Cross dating is a powerful tool for studying growth of freshwater mussels. Fundamental prerequisites for cross dat-

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Table 2. Pearson correlations (with significant relationships ($\alpha < 0.05$) indicated in bold) between master chronologies of 13 southeastern USA mussel species (species abbreviations follow their names), mean annual streamflow, and mean annual air temperature.

Species	EA	EC	FC	FE	LO	OU	PD	QA	QV	LT	PP	QQ	QP
Sipsey River													
Elliptio arca (EA)	**1.00**												
Elliptio crassidens (EC)	**0.63**	**1.00**											
Fusconaia cerina (FC)	**0.48**	**0.53**	**1.00**										
Fusconaia ebena (FE)	**0.73**	**0.74**	**0.62**	**1.00**									
Lampsilis ornata (LO)	**0.47**	**0.50**	**0.44**	0.26	**1.00**								
Obovaria unicolor (OU)	**0.55**	**0.57**	0.25	**0.51**	**0.45**	**1.00**							
Pleurobema decisum (PD)	0.35	**0.47**	**0.67**	**0.49**	0.03	0.31	**1.00**						
Quadrula asperata (QA)	**0.42**	**0.72**	**0.58**	**0.71**	0.23	**0.37**	**0.65**	**1.00**					
Quadrula (Tritogonia) verrucosa (QV)	0.32	0.28	**0.48**	**0.39**	0.31	0.34	0.36	0.37	**1.00**				
St. Francis River													
Lampsilis teres (LT)	**0.69**	**0.76**	**0.72**	0.03	0.53	0.16	0.02	**0.88**	–0.05	**1.00**			
Potamilus purpuratus (PP)	0.46	0.04	**0.76**	0.37	0.49	0.26	0.37	**0.84**	0.64	**0.70**	**1.00**		
Quadrula quadrula (QQ)	**0.60**	0.64	0.52	0.02	**0.48**	0.03	–0.13	**0.75**	–0.33	**0.88**	**0.74**	**1.00**	
Little Tallahatchie River													
Quadrula pustulosa (QP)	**–0.38**	0.02	–0.14	–0.15	–0.16	0.19	–0.14	0.06	–0.38	0.18	0.32	0.39	**1.00**
Abiotic factors													
Mean annual streamflow	**–0.59**	**–0.42**	**–0.41**	**–0.51**	**–0.46**	**–0.38**	–0.23	**–0.35**	–0.40	**–0.60**	**–0.93**	**–0.73**	0.10
Mean annual air temperature	0.08	–0.07	0.09	0.00	–0.14	–0.14	0.06	0.03	0.27	–0.23	0.09	–0.27	–0.03

ing are as follows: (*i*) growth shows substantial non-age-related variation and (*ii*) growth is synchronous among individuals within a population (Black et al. 2005; Helama et al. 2006); the growth patterns that we observed for freshwater mussels satisfy these conditions. After removing age-related variation, growth of all species showed years of high and low growth interspersed among average growth years. Significant mean interseries correlations for all species showed that a high percentage of non-age-related growth variation was shared among individuals within a population. The level of growth synchrony that we observed was similar to or higher than that reported in cross-dating studies for a wide range of other organisms, including marine and freshwater bivalves (Table 3).

Although based on standard and well-defined dendrochronology techniques, cross-dating methods for freshwater mussels are not well established. For example, we found it necessary to iteratively determine optimal spline flexibilities used in our detrending algorithms and these splines varied among mussel species, in contrast to trees which have splines that are often optimized at a flexibility of about 32 years (Grissino-Mayer 2001). However, our sclerochronologies (and many other sclerochronologies) are shorter than most tree-ring chronologies, which are rarely less than 100 years long. Detrending functions differed among mussels likely because of their short chronologies and variability in life span of study species. These results are similar to previous sclerochronological studies with other animals that required case-by-case development of optimal detrending functions (e.g., Helama et al. 2006), highlighting the need for experimental optimization when cross-dating taxa for which little previous guidance exists.

In addition to interpretation of statistical results, such as those provided by COFECHA, validation of shell rings should always be accompanied by a visual or graphical assessment of growth patterns. A combination of visual and mathematical techniques will undoubtedly provide the best cross-dating results. For some individuals, visual cues alone might be sufficient to successfully cross-date a sample even without measurement of growth increments and subsequent statistical analysis (Yamaguchi 1991). In more subtle cases, increment measurement and chronology creation can provide effective cross dating not immediately apparent to the naked eye. However, identification of pointer years, both visually and graphically (here, years where growth index is >|0.5|), is critical for cross dating as they provide natural markers that can be used to validate growth increments and demonstrate growth synchrony. Pointer years also provide information about effects of extreme environmental events on growth and can be used to align measurement series from different individuals, allowing placement in absolute time of specimens with otherwise unknown temporal context (e.g., tree stumps, long-dead mussel shells) (Douglas 1921; Fritts 1971; Helama et al. 2007). In our study, several consistent pointer years were evident, providing reference points for qualitatively checking the alignment of chronologies. Other visual cues potentially useful for cross-dating mussels include disturbance rings and diffuse dark bands. In this study, we do not examine the utility of these markers for cross dating. However, because they occur commonly in mussels, disturbance rings and other visual cues need to be studied more thoroughly to assess their potential as markers for cross dating. Even if natural events sufficient to produce disturbance marks in a high percentage of individuals are rare, less severe events may be recorded in other ways. A mass appearance of diffuse dark bands was observed in 2004 in several mussel species in the Little Tallahatchie River, Mississippi, but not in other years or other rivers (Haag and Commens-Carson 2008). These visual markers along with statistical approaches to cross dating (e.g., those provided by COFECHA) offer an array of tools with which to cross-date and validate rings in freshwater mussel shells.

Cross dating and subsequent analysis of shell rings can be used to validate the assumption of annual deposition of shell

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Table 3. Comparison of interseries correlation coefficients using tree-ring cross-dating techniques for a variety of taxa and ecosystems globally.

Species	Location	Mean or range of interseries *R*	Source
Lake sturgeon (*Acipenser fulvescens*)	Canadian Rivers	0.11	LeBreton et al. 1999
Splitnose rockfish (*Sebastes diploproa*)	Pacific Ocean	0.53	Black et al. 2005
Scots pine (*Pinus sylvestris*)	Scandinavia	0.21–0.58	Helama et al. 2007
Baldcypress (*Taxodium distichum*)	Southeastern USA	0.48	Stahle et al. 1985
White oak (*Quercus alba*)	Quebec	0.42–0.66	Tardif et al. 2006
Red oak (*Quercus rubra*)	Quebec	0.38–0.66	Tardif et al. 2006
Holarctic pearl mussel (*Margaritifera margaritifera*)	Scandinavia	0.39–0.67	Helama et al. 2006
Ocean quahog (*Arctica islandica*)	Scandinavia	0.48	Helama et al. 2007
Southeastern USA mussels	Southeastern USA	0.37–0.96	This study

rings in freshwater mussels. Using cross dating, we showed conclusively that shell growth increments are formed in synchrony among individuals and species and, therefore, that regular formation of growth rings is a pervasive feature of mussel growth. However, synchrony of growth ring formation alone is not necessarily indicative of annual formation of these rings. For all but four of our study species (see next sentence), the annual formation of growth rings has been demonstrated previously and independently using a mark–recapture approach (Haag and Commens-Carson 2008), confirming that the synchronous growth patterns we observed are indeed reflective of annual phenomena. Annual formation of growth rings by *E. crassidens*, *F. ebena*, *O. unicolor*, or *P. decisum* has not been previously validated but is supported by the strong correlation of their master chronologies with the master chronologies of species demonstrated to produce annual rings through mark–recapture. Even without independent confirmation of annual ring formation from mark–recapture studies, the significant correlations of master growth chronologies with annual variation in streamflow for all species except *Q. pustulosa* provide strong evidence that these chronologies and the rings on which they are based reflect annual patterns of growth.

One of the greatest challenges in interpreting mussel shell rings, even after validation, is differentiating annual rings from nonannual rings, particularly those caused by disturbance. Although these rings often have consistent, diagnostic characteristics (Neves and Moyer 1988; Haag and Commens-Carson 2008), differentiation can be challenging, particularly when disturbance rings are superimposed over or located adjacent to annual rings, masking or conflating two independent events in the individual's growth history. Cross dating and subsequent lagging of time series was an effective quality control technique and identified errors in both interpretation of thin sections (e.g., differentiating between annual and nonannual shell rings) and measurement of growth increments. At least 16% of our specimens had some form of interpretive or measurement error, showing that even independent analysis by two experienced readers can result in errors. For this reason, we encourage routine application of cross dating to studies involving mussel shell ring interpretation.

A small percentage of specimens in our study remained unvalidated after quality control. This is common in dendrochronology and can be interpreted as being caused by variable degrees of stress, disturbance, or injury experienced by individuals (Grissino-Mayer 2001). Freshwater mussels likely also experience growth anomalies or trauma that result in deviation from growth synchrony (e.g., predation attempts, flood displacements). Alternatively, specimens could have retained interpretive errors that we were unable to detect even after re-examination. Interpretive errors are a common risk in all growth studies but cross dating minimizes their impact; the agreement of our interseries correlations with a wide variety of other cross-dating studies confirmed the overall robustness and accuracy of our interpretations.

The strong correlations of growth among species within a community, among species in different rivers, and with mean annual streamflow suggest that mussel growth is influenced by pervasive environmental factors. Mussel growth was influenced similarly by streamflow, and potentially other factors, at a regional scale, as the Sipsey River and St. Francis River sites are separated by over 350 km. In contrast, growth of *Q. pustulosa* in the Little Tallahatchie River was not correlated with streamflow or with other species, likely because the dam tailrace habitat is uncoupled from natural variations in streamflow (e.g., Poff et al. 1997). Growth of all other species showed significant or near significant negative correlations, with streamflow indicating that high flow years produce lower growth relative to low flow years. In a previous study, mussel survival was lower in years of extremely high flows (Villella et al. 2004), but virtually nothing is known about the effect of streamflow on freshwater mussel growth. Our findings suggest that streamflow is an important factor in mussel growth; cross dating, followed by analysis of shell ring patterns, will allow investigation of this understudied relationship. Growth of *Margaritifera margaritifera* in Sweden was strongly correlated with summer air temperature, but the strength of this relationship decreased from north to south (Schöne et al. 2004), suggesting that mussels at higher latitudes are limited by availability of warm temperatures favorable for growth. The lack of correlation between temperature and growth for any species in our study suggests that temperature is not a limiting factor for growth in warmwater temperate rivers.

In most organisms, production of distinct growth rings is associated with a strong, predictable annual cycle in some environmental variable(s) (Black et al. 2005; Tardif et al. 2006). Growth rings in temperate-zone trees, fishes, and

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marine bivalves are produced during periods of growth cessation in winter (LeBreton and Beamish 2000; Schöne et al. 2002; Rypel et al. 2006), whereas growth rings in tropical treeline trees are produced in response to slow growth in the dry season (Biondi 2001). Distinct nonannual tree rings (e.g., disturbance rings) can occur in all individuals in a population in response to some major environmental event (e.g., fire, drought), but such rings are sporadic and irregular in occurrence (Payette et al. 1990; Barber et al. 2000; Kulakowski and Veblen 2007). Regularly occurring intraannual rings such as spawning checks in fishes or bivalves are usually readily distinguishable from more distinct rings formed at the end of the growing season (Jones 1980; Campana 2001). For these reasons, formation of distinct annual growth increments is a nearly universally held tenet for many organisms (Campana and Thorrold 2001) and validation of this assumption is often accomplished and accepted on the basis of cross dating alone (Black et al. 2005). A growing body of evidence supports the production of annual winter rings in a taxonomically wide variety of freshwater mussels from a wide variety of habitats (e.g., Mutvei and Westermark 2001; Valdovinos and Pedreros 2007; Haag and Commens-Carson 2008), opening the possibility that cross dating can be used as a stand-alone technique for validation of shell rings.

Widespread validation of annual shell rings in mussels has not taken place, thus the strongest validation approach remains utilization of a mark–recapture study with cross dating to provide independent evidence for annual periodicity and identification of interpretive errors. However, the requirement of an independent mark–recapture study can be obviated in some cases. First, if annual ring formation has been validated in other species in an assemblage or region, a high correlation between master chronologies of these species with unstudied species provides strong evidence for annual ring formation in the unstudied species. Second, even if no information exists for other species in an area, a high correlation between putative annual growth increments and a strong, variable environmental signal (e.g., annual streamflow) can provide convincing support for the annual formation of shell rings. We used cross dating to show that freshwater mussels produce annual rings, individuals within and among populations show synchronicity of growth, and mussel growth is negatively correlated with mean annual streamflow. Although cross dating has rarely been applied to studies of freshwater mussel growth, its use will help unlock the vast information contained within shell rings.

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Comparison of three microquantity techniques for measuring total lipids in fish

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Abstract: To measure lipids in juvenile and adult fishes, we refined three microquantity approaches (microgravimetric, microcolorimetric sulfophosphovanillan (SPV), and Iatroscan thin layer chromatography – flame ionization detection (TLC–FID)) that were originally developed to measure lipids in small aquatic invertebrates. We also evaluated their precision and comparability by quantifying the total lipid content of age-1+ walleye (*Sander vitreus*), yellow perch (*Perca flavescens*), and lake whitefish (*Coregonus clupeaformis*) collected in Lake Erie (US–Canada), Lake Michigan (USA), and Muskegon Lake (Michigan, USA). Our findings demonstrate that (*i*) microquantity approaches provide estimates of total lipids in juvenile and adult fishes similar to those of more traditional macroquantity approaches, (*ii*) the microcolorimetric SPV and microgravimetric approaches produce near identical estimates of total lipid content, and (*iii*) the Iatroscan TLC–FID approach underestimates total lipids relative to the other approaches for individuals with high lipid levels. Ultimately, our research makes available additional techniques for measuring total lipid content of fishes that are less expensive than traditional techniques, owing to a reduced need for large quantities of samples and solvents.

Résumé : Afin de doser les lipides dans les poissons juvéniles et adultes, nous raffinons trois méthodologies microquantitatives (microgravimétrie, microcolorimétrie à sulfophosphovanilline (SPV) et chromatographie sur couche mince Iatroscan couplée à un détecteur à ionisation de flamme (TLC–FID)) mises au point au départ pour mesurer les lipides dans de petits invertébrés aquatiques. Nous avons évalué leur précision et les avons comparées entre elles en dosant le contenu en lipides totaux de dorés (*Sander vitreus*), de perchaudes (*Perca flavescens*) et de grands corégones (*Coregonus clupeaformis*) d'âge 1+ récoltés aux lacs Érié (É.-U. – Canada), Michigan (É.-U.) et Muskegon (Michigan, É.-U.). Nos observations démontrent que (*i*) les méthodes microquantitatives fournissent des estimations des lipides totaux chez les poissons juvéniles et adultes semblables à celles des méthodologies macroquantitatives plus courantes, (*ii*) les méthodologies microcolorimétriques SPV et microgravimétriques donnent des estimations presque identiques du contenu lipidique total et (*iii*) la méthodologie TLC–FID Iatroscan sous-estime les lipides totaux par comparaison aux autres méthodes chez les individus qui ont un fort contenu lipidique. En fin de compte, notre recherche rend disponibles de nouvelles techniques pour mesurer le contenu lipidique total chez les poissons, qui sont moins coûteuses que les techniques habituelles, parce qu'elles requièrent de moins grandes quantités d'échantillons et de solvants.

[Traduit par la Rédaction]

Introduction

Lipids are an integral component of aquatic animals, and their estimation can provide insight into growth strategies, health and condition, and survival potential of aquatic invertebrates and vertebrates alike (e.g., Arts et al. 1993; Ludsin and DeVries 1997; Post and Parkinson 2001). In turn, numerous approaches for quantifying lipid content of aquatic biota have been developed. Older, more traditional lipid analyses (e.g., Folch et al. 1957; Bligh and Dyer 1959) require large quantities of both the sample and solvents (e.g., methanol, chloroform), which can be problematic when dealing with organisms with low lipid levels (e.g., pooling of individuals is required; see Miranda and Hubbard 1994) or a small budget. However, with improved technological and analytical capabilities, "microquantity" approaches that require only micrograms of sample and microlitres of solvents have been developed for estimating lipid content of aquatic organisms. In turn, sample processing costs have declined and the ability to assess variation in lipid content at the level of an individual has increased.

There are three microquantity techniques that have be-

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come commonly used in the aquatic sciences: (*i*) Van Handel (1985) developed a microquantity colorimetric sulfo-phosphovanillan (SPV) method to measure total lipids in mosquitoes; (*ii*) Gardner et al. (1985) developed a microgravimetric assay to measure total lipids in a variety of freshwater aquatic invertebrates (e.g., amphipods, oligochaetes); and (*iii*) Parrish (1986, 1987) introduced a microquantity thin-layer chromatographic approach, using an Iatroscan flame ionization detection system (Iatroscan TLC–FID; Mitsubishi Kagaku Iatron, Inc., Nishi-Goken-cho, Shinjuku-ku, Tokyo, Japan) to measure lipid classes (e.g., triglycerides, sterol esters, sterols, free fatty acids, phospholipids) and total lipids (by summing lipid classes), which has been adapted for use with zooplankton, benthic macroinvertebrates, larval fish, and juvenile fish (Fraser et al. 1985; Arts et al. 1993; Ludsin and DeVries 1997).

Despite development of an array of microquantity approaches for use with small aquatic organisms, not all techniques, including Van Handel's (1985) microcolorimetric SPV approach and Gardner et al.'s (1985) microgravimetric approach, have been adapted for use with comparatively large organisms such as juvenile and adult fish. Further, our understanding how these three microquantity techniques perform relative to one another is incomplete. Gardner et al. (1985), however, did find that their microgravimetric assay correlated strongly with Iatroscan TLC–FID measurements for individual amphipods and oligochaetes. Further, although Van Handel's (1985) microcolorimetric SPV approach has been preferred over Gardner et al.'s (1985) microgravimetric method for measuring total lipids of benthic macroinvertebrates, because it is more time- and labor-efficient (Landrum et al. 2002, 2004; Inouye and Lotufo 2006), a true comparison of the relative performance of these two microquantity methods has not yet been conducted for any organism.

Owing to differences in how lipids are quantified among gravimetric, colorimetric SPV, and Iatroscan TLC–FID techniques, a potential exists for method-specific biases. For example, gravimetric approaches have potential to be biased upwards because of inclusion of nonlipid materials (Hopkins et al. 1984). By contrast, colorimetric SPV approaches, which measure the absorbance of the red–purple complex produced from the reaction between double carbon bonds and the SPV reagent, may be biased downwards, as only compounds containing unsaturated carbon bonds are quantified (Barnes and Blackstock 1973). Finally, the Iatroscan TLC–FID approach, which sums individual lipid classes to generate an estimate of total lipids, has potential to underestimate lipids, owing to nonlinear responses of the TLC–FID detector (e.g., Parrish 1986, 1987). If reliable comparisons among studies employing these microquantity techniques to quantify total lipids are to be made, a critical evaluation of their relative performance is needed.

Herein, we conducted a laboratory study designed to determine how these microgravimetric, microcolorimetric SPV, and Iatroscan TLC–FID approaches compare with one another in terms of measuring total lipid content of three fishes. In so doing, we developed protocols for using Gardner et al.'s (1985) microgravimetric and Van Handel's (1985) microcolorimetric SPV approaches for use with juvenile and adult fishes. We also refined Parrish's (1986, 1987) protocol and provided an alternative to Ludsin and DeVries' (1997) protocol for using Chromarod Iatroscan TLC–FID to measure total lipids (by summing lipid classes) of juvenile and adult fishes. Ultimately, through development of these protocols and comparison of the relative performance of these techniques, we sought (*i*) to enhance the abilities of scientists to conduct inexpensive, yet reliable, analyses of lipid content in fishes, and (*ii*) to provide a means to compare findings from previous investigations that used any of these methods for measuring total lipids.

Materials and methods

Sample preparation

We analyzed the whole-body lipid content of age-1+ walleye (*Sander vitreus*), yellow perch (*Perca flavescens*), and lake whitefish (*Coregonus clupeaformis*), which are of economic and ecological importance in numerous freshwater systems throughout North America and represent a wide range of lipid levels in fish. Samples were collected from Lake Erie, Lake Michigan, or Muskegon Lake (a drowned-river mouth located at Muskegon, Michigan). We also analyzed lipid content of individual gonads (testes = 4, ovaries = 6) from a subset of 10 walleye, which originated in western Lake Erie but were being reared in the Michigan Department of Natural Resources' Saline Fish Hatchery (Saline, Michigan). All other fish were collected during May to September of 2005 or June to October of 2006 via bottom trawling (Table 1). Upon collection, fish were measured (to the nearest millimetre total length (TL)) and weighed (to the nearest gram). After fish reached a frozen consistency in the freezer (the grinder performs better on frozen material), they were immediately homogenized with a Waring Commercial blender (model HGB-300, Waring Corp., New Hartford, Connecticut, USA) with no additional liquid and stored in individually labeled glass jars at –80 °C until being freeze-dried. Freeze-dried samples were then reground with a Hamilton Beach hand blender (model 59775, Hamilton Beach Co., Glen Allen, Virginia, USA) and sifted through a 1.68 mm (no. 12) sieve. Although the average particle size was much smaller than 1.68 mm, this sieve was the smallest one that would allow the sample to readily pass through it (owing to clumping of lipid-rich particles), while still removing the few remnants of larger bone or scale. Samples were then stored at –80 °C until lipid analysis.

To verify that our microquantity methods were producing total lipid values on par with those of more traditional macroquantity methods, we first compared total lipid content of 19 randomly selected individuals (across all three species collected during 2006) using Folch et al.'s (1957) macroquantity method and Van Handel's (1985) microquantity colorimetric SPV method. We then compared total lipid values and precision of these estimates among the microgravimetric, microcolorimetric, and Iatroscan TLC–FID methods. All reagents used in this entire study were HPLC grade. We described the methods for all four techniques below.

Macroquantity method

Our macroquantity procedure followed that of Folch et al. (1957) with slight modifications. Each sample was run at least in triplicate, with solvent blanks also being analyzed.

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Table 1. Summary statistics for fish samples analyzed in this study.

Species	Tissue	Collection lake	Collection period	TL (mm)	Mass (g)	Lipid (%)	CV (%)	*N*	Comparison
Walleye	Body	Hatchery (Erie)	April 2005	576±25	1758±321	27.0±5.2	8.7±4.5	10	SPV vs. MG
	Gonad	Hatchery (Erie)	April 2005	NA	200±225	24.7±12.8	8.8±4.2	10	SPV vs. MG
	Body	Muskegon, Michigan	June–October 2006	461±106	1103±784	33.2±8.6	8.9±6.3	26	SPV vs. TLC–FID
									SPV vs. Macro (N = 8)
Yellow perch	Body	Erie	May–September 2005	247±32	185±65	20.2±4.4	10.3±6.9	10	SPV vs. MG
	Body	Muskegon, Michigan	June–October 2006	266±33	235±91	22.1±5.6	8.9±6.5	18	SPV vs. TLC–FID
									SPV vs. Macro (N = 3)
Lake whitefish	Body	Michigan	June–October 2006	501±51	1130±298	28.6±8.8	7.2±5.2	41	SPV vs. TLC–FID
									SPV vs. Macro (N = 8)

Notes: Walleye, *Sander vitreus*; yellow perch, *Perca flavescens*; lake whitefish, *Coregonus clupeaformis*. Samples were collected in Muskegon Lake, Lake Michigan, and Lake Erie during 2005 and 2006. The average total length (TL ±1 standard deviation (SD)), wet mass (Mass, ±1 SD), percent lipids (Lipid, ±1 SD), and coefficient of variation (CV, ±1 SD) are reported, as are sample sizes (N). Percent lipids and CVs were determined from the microcolorimetric SPV method, as it was the only method used on all samples. We also indicate the comparative analyses conducted with each data set (SPV, microcolorimetric; TLC–FID, Iatroscan; MG, microgravimetric; Macro, macroquantity). NA, not applicable.

We extracted lipids from a 1 g subsample of homogenized freeze-dried tissue in a 20 mL chloroform–methanol mixture (2:1 by volume), using a 30 mL Wheaton tissue homogenizer with a Teflon pestle that was powered by a household drill (2500 r/min (1 r = 2π rad)). The homogenization was conducted in an ice bath to prevent evaporation. After the mixture was agitated thoroughly using a vortex, it was decanted into a 50 mL centrifuge tube and centrifuged at 2400 r/min for 15 min, which caused the solid and the liquid phases to separate. The liquid (lipid) phase was recovered into a graduated 50 mL centrifuge tube, using a pipette, to which 0.9% NaCl solution (0.2 volume of lipid phase) was added. The lipid–chloroform solution was then vortexed twice for ~5 s each time and centrifuged at 2400 r/min for 5 min to further purify the lipid phase.

After recording the volume of the lower chloroform phase (V_p), 5 mL (V_s) of it was transferred into preweighed vials using a graduated pipette. The vials were dried overnight at ~50 °C, while being purged continuously with nitrogen gas, until a constant mass was achieved. The percent dry mass of lipids (% lipid) was calculated as

$$(1) \qquad \% \text{ lipid} = \frac{(W_m - W_b)V_p}{V_s M_f} \times 100$$

where W_m is the sample lipid mass (mg) and W_b is the mass of "lipids" from blanks (mg), V_p is the volume (mL) of the chloroform phase after NaCl purification and centrifuging, V_s is the volume of the lipid subsample (i.e., 5 mL in this method), and M_f is the dry mass of fish (mg).

Microquantity methods

Microcolorimetric SPV and Iatroscan TLC–FID lipid extraction and purification

The microquantity SPV and Iatroscan TLC–FID analyses were conducted on the same day, using the same lipid extracts. The procedure for extracting total lipids from our freeze-dried samples for these two microquantity approaches followed that of Gardner et al. (1985), with some modifications to improve the efficiency of time and labor. A 1 mg "control" extract was made from artificial plankton (Argent Chemical Laboratories, Redmond, Washington, USA), and blanks were taken through the entire procedure.

To extract lipids, a subsample of 1–3 mg (measured to nearest 0.001 mg) was measured into glass test tubes (50 mm × 6 mm), and 200 µL of the 2:1 chloroform–methanol extraction solvent was added to each sample. Samples were homogenized for 30 s at 5000 r/min using a variable-speed rotary tool (Dremel model 380-5) with a glass bead pestle. This homogenate was decanted into a 1.5 mL conical centrifuge tube. Subsequently, another 200 µL of the chloroform–methanol extraction solvent was added to each centrifuge tube and the mixture was immediately homogenized briefly (~5 s), on ice, to prevent evaporation.

To purify the lipid extract, 200 µL of a 0.9% NaCl solution was added to each sample, which then was mixed by vortexing. Centrifuge tubes were then placed in a centrifuge (model 5415, Eppendorf, Hamburg) for 15 s at 8000 r/min, or a relative centrifugal force of 5220*g*. At this force, the phase separation occurs almost immediately and increasing time did not improve separation in controlled trials. This

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phase separation resulted in nonlipid compounds and methanol being partitioned into the upper aqueous phase and the lipid–chloroform extract being partitioned into the lower organic phase. From this organic lower phase, a microsampling pipette was used to remove 100 and 3 μL aliquots for microcolorimetric SPV analysis and Iatroscan TLC–FID analysis, respectively.

Microcolorimetric SPV analysis

Our microcolorimetric SPV analysis was conducted on all samples (both gonads and fish) collected during 2005 and 2006 (see Table 1), with all samples analyzed in triplicate at a minimum. Our methods followed those of Van Handel (1985).

Culture tubes (13 mm × 100 mm) containing 100 μL of lipid extract were heated at 100 °C for 15 min on a dry bath heater to remove the chloroform solvent. We made certain to complete evaporation of the chloroform as it has a positive interference with the phosphovanillin reagent. We added 0.25 mL of 96% H_2SO_4 to the bottom of the culture tube to make complete contact with the lipid residue, and samples were then vortexed and heated for 10 min at 100 °C on a dry bath heater. We added 5 mL of the colorimetric SPV reagent (consisting of 3 g vanillin, 0.5 L deionized water, 2 L concentrated phosphoric acid, stored in the dark) to each tube and thoroughly mixed tube contents by vortexing.

After incubating at room temperature for 30 min, the absorbance (A, nm; measured at 525 nm) was measured using a Turner spectrophotometer (model SP-870, Barnstead, Dubuque, Iowa). Solvent blanks were run through the entire procedure and used to "zero" the spectrophotometer. We used pure olive oil (10 mg·mL^{-1}) in the 2:1 chloroform–methanol solvent mixture as a stock standard (Knight et al. 1972; Ahlgren and Merino 1991). Working volumes of 0 (blank), 10, 20, 30, 40, and 50 μL of this standard solution were used to develop a standard curve for lipids (absorbance vs. mass (mg) of the standard), with the percent dry mass of lipids (% lipid) calculated as follows:

$$(2) \qquad \%\ \text{lipid} = \frac{AV_p}{V_s S_s M_t} \times 100$$

where V_p, V_s, and M_t are as described earlier (V_s = 100 μL in this method) and S_s is the slope of the regression line of the stock standard (nm/mg).

Iatroscan TLC–FID analysis

Individual lipid classes, which were summed into a total lipid estimate, were quantified on all fish collected during 2006 (see Table 1), with all samples run in triplicate. We followed Parrish's (1986) protocol, with slight modifications.

Iatroscan rods were spotted just below the origin, but well above the solvent level, with 3 μL of the same organic-phase extracts used for microcolorimetric SPV analysis. All developments were performed in 100 mL of solvent in 15 cm × 18 cm TLC development tanks. Developments were modified so as to allow for a single scan of the entire rod length (Ludsin and DeVries 1997). Specifically, we developed rods in a chloroform–methanol–water solution (5:4:1 by volume) until the leading edge of the solvent reached 1 cm above the origin. The rods were then developed in a hexane – diethyl ether – formic acid solution (99:1:0.05 by volume) for 52 min. Rods were then developed in a hexane – diethyl ether – formic acid solution (80:20:0.1 by volume) for 42 min. This scheme allowed for the separation of seven lipid peaks (i.e., hydrocarbons, sterol esters and waxy esters, triglycerides, free fatty acids, alcohols, sterols, and phospholipids) and allowed for maximum separation of the most abundant lipids (i.e., triglycerides, free fatty acids, and phospholipids). Prior to each development, rods were air-dried for 5 min and conditioned in a constant humidity chamber (~30%) that was saturated with aqueous $CaCl_2$.

Individual lipid classes were measured with an Iatroscan Mark IV (Iatron Laboratories, Tokyo, Japan) connected to a windows-based computer running Peak Simple Chromatography software (Shell USA, Fredericksburg, Virginia). The hydrogen flow rate was set at 180 mL·min^{-1} and scan rate was set at 4 mm·s^{-1}. Rods were analyzed in racks of 10 comprised of three samples in triplicate and an accompanying seven-lipid component standard consisting of hydrocarbons (squalene), sterol esters (cholestryl tridecanoate), triacylglycerols (tripalmitin), free fatty acids (palmitic acid), aliphatic alcohols (cetyl alcohol), alicyclic sterols (cholesterol), and phospholipids (phosphatidylcholine). To ensure cleanliness of rods, all rods were blank-scanned immediately prior to spotting with sample or standards. Subsequent to analysis, individual lipid classes were summed to estimate total lipids (W_m). The total lipid percents were calculated as follows:

$$(3) \qquad \%\ \text{lipid} = \frac{W_m V_p}{V_s M_t} \times 100$$

where W_m, M_t, V_p, and V_s (i.e., 3 μL in this method) are as described earlier.

Microgravimetric analysis

Because microgravimetric analysis is more time- and labor-intensive than microcolorimetric SPV analysis (Landrum et al. 2002, 2004; Inouye and Lotufo 2006), it was conducted only on samples collected during 2005. These samples included yellow perch, walleye, and walleye gonads (Table 1). Each sample was run in triplicate at a minimum. Our microgravimetric analysis followed Gardner et al.'s (1985) procedure with slight modifications. A 1 mg "control" extract was made from artificial plankton (Argent Chemical Laboratories), and blanks were taken through the entire procedure.

To extract lipids, 100 μL of a 2:1 chloroform–methanol extraction solvent was added to a 0.3 to 1.3 mg subsample of freeze-dried tissue and homogenized 30 s at 5000 r/min. The homogenate was then drawn into a borosilicate glass microcapillary pipette to a height of no more than 65 mm, and the lower tip of the pipette was sealed with a moist inorganic clay preparation. The pipettes were shortened to fit into an IEC–MB microhematocrit centrifuge (International Equipment Company, Needham Heights, Massachusetts, USA), which was run for 1 min at 13 500 r/min to remove any nonlipid particles. The supernatant was then transferred into clean test tubes with a calibrated capillary pipette.

To generate a purified lipid extract of known quantity, we

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first washed the crude lipid extract by adding 20% (by volume) of a 0.9% NaCl solution and next transferred it into a clean 100 µL capillary pipette. We then purified it by centrifuging for 1 min, discarding the aqueous, nonlipid layer. A known volume (V_s) of this subsample was transferred to a set of tared aluminum weighing tins, the solvent was evaporated on a heater (Themolyne Dri-Bath) set at 60 °C for 15 min, and the remaining lipids were then weighed. We calculated the percent dry mass of lipids (% lipid) as in eq. 1.

Statistical analysis

Because the microcolorimetric SPV analysis was the only technique conducted on all samples, all comparisons are made to this technique. Specifically, we compared the percent total lipid and coefficient of variation (CV = (sample standard deviation / sample mean) × 100) between (*i*) the microcolorimetric SPV and macroquantity techniques (n = 19 random samples from 2006), (*ii*) the microcolorimetric SPV and the microgravimetric methods (using 2005 data), and (*iii*) the microcolorimetric SPV and the Iatroscan TLC–FID methods (using 2006 data) (Table 1). For each comparison, we determined whether average total lipids for individuals significantly differed from a 1:1 line (i.e., slope = 1; intercept = 0) and also conducted an analysis of covariance (ANCOVA) to determine whether this relationship differed among species or sample groups: (*i*) walleye, yellow perch, and lake whitefish for microcolorimetric SPV vs. macroquantity methods; (*ii*) walleye, walleye gonads, and yellow perch for microcolorimetric SPV vs. microgravimetric methods; and (*iii*) walleye, lake whitefish, and yellow perch for the microcolorimetric SPV vs. Iatroscan TLC–FID methods (see Table 1). To compare measurement variability (i.e., CVs) between techniques (i.e., microcolorimetric SPV vs. macroquantity; microcolorimetric SPV vs. microgravimetric; microcolorimetric SPV vs. Iatroscan TLC–FID), we conducted paired t tests with all samples combined (all species and locations). We also conducted ANCOVAs for these comparisons with species and microquantity technique (per above) as independent factors.

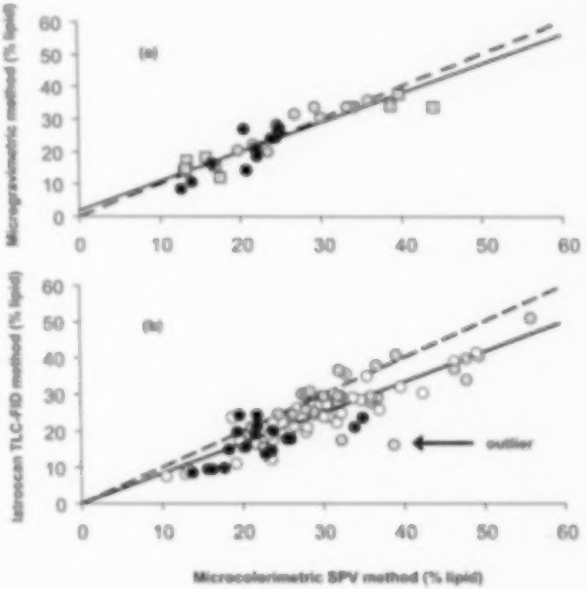
After each regression analysis, we conducted a residual analysis to identify outliers (i.e., observations with standardized residuals > |3.5|; Faraway 2005). Only one outlier was identified (a 2006 walleye; standardized residual = –3.6) and removed from analyses. No data transformations were required as all data were normal (Kolmogorov–Smirnov normality tests; all $p > 0.05$) and variances were homogeneous (F tests; all $p > 0.05$). Our α level was set at 0.05 for all tests.

Results

Microcolorimetric SPV method vs. macroquantity method

The microcolorimetric SPV method provided estimates of total lipid content similar to those of Folch et al.'s (1957) macroquantity method. Specifically, we found a strong positive relationship between methods (R^2 = 0.82; F = 76.97, $p < 0.0001$), with the slope not different from one (95% confidence interval (CI) of slope = 0.98 to 1.60) and the intercept not different from zero (p = 0.59, 95% CI of intercept = –10.03 to 5.89). This relationship also did not vary among species (i.e., lake whitefish, walleye, yellow perch), nor did we find a species–method interaction (species effect, F = 0.13, p = 0.88; species–method interaction effect, F = 0.26, p = 0.77). By contrast, the average CV ± 1 standard deviation (SD) (3.2% ± 1.9%) of the macroquantity method was significantly lower than for the microcolorimetric SPV method (9.4% ± 7.4%) (paired t test, F = 20.36, p = 0.002). Thus, although the microcolorimetric SPV and macroquantity methods produced similar total lipid estimates, the precision in these estimates was better with the macroquantity approach than with the microcolorimetric SPV approach.

Fig. 1. Relationship between the microcolorimetric sulfophosphovanillan (SPV) and (*a*) microgravimetric method and (*b*) Iatroscan thin layer chromatography – flame ionization detection (TLC–FID) methods for estimating total lipid content (percent lipids) in fish tissue. Symbols: open circles, lake whitefish; solid circles, yellow perch; shaded circles, walleye; shaded squares, walleye gonad; solid line represents the regression line; broken line represents the y–x (1:1) line. The one outlier removed from this study is identified in *b*.



Microcolorimetric SPV method vs. microgravimetric method

Regression analysis indicated no difference in the ability of the microcolorimetric SPV and microgravimetric methods to measure total lipid content. The relationship between methods was positive and strong (R^2 = 0.88; F = 129.0, $p < 0.0001$; Fig. 1*a*), the slope was not different from one (95% CI of slope = 0.74 to 1.1), and the intercept did not differ from zero (p = 0.38; 95% CI of intercept = –2.3 to 5.9). Likewise, ANCOVA revealed no effect of sample type (i.e., walleye, walleye gonads, yellow perch) on this relationship (F = 2.0, p = 0.15) or a sample–method interaction (F = 2.4, p = 0.11).

Coefficients of variation ranged from 0.5% to 20.5% for

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the microcolorimetric SPV method and from 1.4% to 25.0% for the microgravimetric method, with respective means (±1 SD) of 9.3% ± 5.2% and 11.5% ± 5.3%. In turn, no difference in measurement variability was evident between analytical techniques (paired t test, $t = 2.0$, $p > 0.5$), and ANCOVA revealed no effect of species or sample type ($F = 0.5$, $p = 0.62$) or species or sample – method interaction ($F = 0.3$, $p = 0.74$).

Microcolorimetric SPV method vs. Iatroscan TLC–FID method

We found a strong positive relationship between the microcolorimetric SPV and Iatroscan TLC–FID methods ($R^2 = 0.78$; $F = 292.0$, $p < 0.0001$) and also found that the intercept of the regression line did not differ from zero ($p = 0.68$; 95% CI of intercept = –3.6 to 2.4). However, the slope of the relationship was significantly less than one (slope = 0.86; 95% confidence intervals of slope = 0.76 to 0.96), indicating that the Iatroscan TLC–FID method underestimated total lipids relative to the microcolorimetric SPV method and that the magnitude of this underestimation increased with increasing lipid content (Fig. 1*b*). There was no effect of species (i.e., walleye, lake whitefish, yellow perch) on this relationship ($F = 1.3$, $p = 0.29$) or a species–method interaction ($F = 1.1$, $p = 0.34$).

No difference in average CVs was evident between techniques (paired t test, $t = 1.6$, $p = 0.11$; ANCOVA: species effect, $F = 1.2$, $p = 0.29$; method effect, $F = 1.3$, $p = 0.25$; species–method interaction, $F = 0.4$, $p = 0.69$). Coefficients of variation ranged from 0.6% to 26.7% for the microcolorimetric SPV method and from 0.3% to 24.6% for the Iatroscan TLC–FID method, with respective means (±1 SD) of 8.1% ± 5.8% and 7.8% ± 4.9%.

Discussion

Microcolorimetric SPV method vs. macroquantity method

Our comparison of Van Handel's (1985) microcolorimetric SPV method and Folch et al.'s (1957) macroquantity method, the latter of which has been widely accepted as a reliable technique with which to measure total lipid for fish (e.g., Rinchard et al. 2007; Nanton et al. 2007), indicates that microquantity and macroquantity approaches can estimate total lipid content of juvenile and adult fishes equally well, as no statistical differences between methods were found in our study. Although this finding may not hold for all microquantity methods (e.g., Iatroscan TLC–FID; see below) and does counter Inouye and Lotufo's (2006) finding that Van Handel's (1985) microcolorimetric SPV method consistently produced higher lipid estimates than their macroquantity method (i.e., Bligh and Dyer 1959), we are encouraged by the strong correspondence between results achieved with the two methods used in our study and recommend continued use of microquantity methods in most circumstances.

However, in instances when low within-sample measurement precision is critical, investigators may want to consider using macroquantity approaches, given that we found macroquantity measurement precision (CVs < 5%) to be superior to microquantity precision (CVs from 20% to 30%). These differences are likely due to a combination of nonhomogeneity in tissue subsamples (at the microscale) and the small subsample volumes of tissue analyzed in our microquantity approaches (i.e., 100 µL aliquots were analyzed for both the microcolorimetric SPV and microgravimetric methods, whereas 3 µL aliquots were analyzed for the Iatroscan TLC–FID method) versus our macroquantity approach (5 mL aliquots). We can only imagine that the differences between microquantity and macroquantity estimates of precision would be heightened had we not sieved samples for large pieces of bone and scales. Thus, investigators seeking high precision in their microquantity estimates of total lipids should (*i*) ensure that samples are finely homogenized, (*ii*) increase within-sample replication, or (*iii*) increase the volume of subsamples analyzed (which may defeat the purpose of using microquantity approaches).

Microcolorimetric SPV method vs. microgravimetric method

Previous investigations have shown a tendency for gravimetric approaches to overestimate lipid levels (Hopkins et al. 1984) and for colorimetric SPV approaches to underestimate them (Barnes and Blackstock 1973). However, we found no evidence to this effect in our study for these two microquantity approaches; we also did not find that the ability to estimate lipids between methods differed among species of varying lipid content or between tissue types (somatic vs. gonadal). Landrum et al. (2002) also demonstrated that the microcolorimetric SPV and microgravimetric methods produce comparable results for aquatic algae. Additionally, measurement precision did not differ between microquantity methods in this study, regardless of species or tissue type analyzed. Although we recommend use of the microcolorimetric SPV approach in future studies, as it has been shown to be more time- and labor-efficient than the microgravimetric approach (Kukkonen et al. 2004; Landrum et al. 2004; Inouye and Lotufo 2006), the lack of difference between these two microquantity approaches for a organisms of both low (algae) and high (somatic and gonadal fish tissue) total lipid content indicates that comparison of studies using these two methods can be conducted with little concern for potential bias.

Importantly, however, this latter conclusion also assumes that appropriate protocols are used to minimize the potential for bias. In this study, we provided three major modifications to extant protocols that we feel improved the accuracy of our total lipid measurements. First, because gravimetric methods measure all compounds in an extract (including potentially nonlipid materials), they have the tendency to overestimate lipid levels (Folch et al. 1957; Gardner et al. 1985). To minimize the potential for this bias, we included a saline (0.9% NaCl) wash to purify the extract (Barnes and Blackstock 1973; Gardner et al. 1985; our study). Although we did not conduct trials without this saline wash to quantify the amount of error that discarding this step would induce (but see Folch et al. 1957), the fact that our lipid estimates did not differ between our microgravimetric and microcolorimetric SPV approaches indicates that this step may be essential. Second, we used concentrated H_2SO_4 in the microcolorimetric SPV procedure to reduce the potential for our colorimetric method to underestimate lipid content.

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which can be problematic because this approach only measures compounds that contain unsaturated bonds (Knight et al. 1972; Barnes and Blackstock 1973). The saturated lipids in our fish tissue, when heated with concentrated H_2SO_4, could most likely generate intermediate, unsaturated products (e.g., ketones) that could react with colorimetric SPV reagents (Ahlgren and Merino 1991). Third, we purified the lipid extract (by adding a saline rinse) in the microcolorimetric SPV procedure. Although previous studies have suggested that this purification step is unnecessary (Barnes and Blackstock 1973; Ahlgren and Merino 1991), preliminary protocol-development trials (n = 5 individuals measured in triplicate) demonstrated that the comparability of our microcolorimetric SPV and microgravimetric methods increased when a purification step was used for both approaches (D. Fanslow, unpublished data). Further, in contrast to Barnes and Blackstock (1973), who found nonlipid materials to have negligible impacts on colorimetric SPV measures, we found that taking pure glycogen and protein through the whole lipid extraction and microcolorimetric SPV quantification procedure resulted in measured "lipid" levels of 9.4% and 1.04% (g/g dry mass), respectively (D. Fanslow, unpublished data). Thus, nonlipid materials can bias microcolorimetric SPV estimates high, and total lipid estimates generated with protocols that lack a purification step should be treated with caution. A final reason for including a purification step in the microcolorimetric SPV technique is to remove residual fish tissues left behind in the homogenization and extraction process. The microcolorimetric SPV method initially developed by Van Handel (1985) was specifically designed for mosquitoes, the tissues of which are too small to interfere with the readings of absorbance values. For large organisms such as fish, however, we found that the solid traces turned brown after heating with concentrated sulfuric acid and absorbance values consequently became unstable.

Microcolorimetric SPV method vs. Iatroscan TLC–FID method

No differences in analytical precision (CVs) were found between the microcolorimetric SPV and Iatroscan TLF–FID techniques. We did, however, find that the Iatroscan TLC–FID approach yielded lower total lipid values than the microcolorimetric SPV method and that these differences increased with increasing lipid content. Although no comparisons were conducted between the microgravimetric and Iatroscan TLC–FID methods, we also expect that the Iatroscan TLC–FID method would underestimate lipids relative to the microgravimetric approach (especially at high lipid levels), given the similarity between the microgravimetric and microcolorimetric SPV approaches (per above).

Underestimation by the Iatroscan TLC–FID method may result from multiple factors. First, although the Mark IV model drastically improved linearity of the FID detector response than the earlier version, some lipids (e.g., cholesterol, monacylglycerol, glycolipids) still generate a curvilinear relationship with sample loads (Parrish 1986; Parrish et al. 1996; Indrasena et al. 2005). Further, this nonlinearity can be exacerbated when microquantities of lipid material (such as used in this study) are used (Kaitaranta and Nicolaides 1981; Ohman 1997; Indrasena et al. 2005), resulting in a weaker FID response that can lead to underestimation of lipids. Second, decomposition of lipid components during sample analysis could affect the accurate measurement of individual classes of lipid on TLC–FID but might not measurably bias the microcolorimetric SPV method. For example, the degradation of some monoacylglycerol polar lipids (e.g., P-choline, P-ethanolamine), which is signified by an increase in free fatty acids, was observed in Iatroscan TLC–FID analysis of both fish and *Artemia* (brine shrimp) lipids (Bligh and Scott 1966; Braddock and Dugan 1972; Sasaki and Capuzzo 1984). Moreover, although methanol is essential for lipid extraction, it also is considered an undesirable solvent because it can react with samples after extraction. Esterification of carboxylic groups with methanol has been considered a serious problem in extracts of airborne particulate (Clement et al. 1982). The presence of methyl esters in the lipid extracts of benthic invertebrates was attributed to the reaction of lipid components with the methanol during sample analysis (Gardner et al. 1985).

Previous studies with aquatic invertebrates have drawn similar conclusions as our own, finding that the Iatroscan TLC–FID methods tend to underestimate total lipids (Fraser et al. 1985; Parrish 1986, 1987). For example, Sasaki and Capuzzo (1984) found that the slope of the relationship between total lipids measured with a macro-Iatroscan TLC–FID approach and macrogravimetric approach was 0.88 for *Artemia* extracts, which was quite similar to that found in our study (0.86). Unfortunately, additional details about Sasaki and Capuzzo's (1984) relationship were not provided. Similarly, Gardner et al. (1985) found that micro-Iatroscan TLC–FID measurements of total lipids were generally lower than microgravimetric measurements of oligochaetes. Given that underestimation of total lipids by Iatroscan TLC–FID approaches appears general to both aquatic invertebrates and fishes, we recommend that this approach only be used to estimate lipids of individual classes, cautioning against the common practice of summing up these results to measure total lipids. Additionally, owing to the potential for Iatroscan TLC–FID to underestimate total lipids in "fatty" fishes, care also should be taken when using lipid values from previous studies that used Iatroscan TLC–FID to quantify total lipids (e.g., Scott et al. 2002; Zhou et al. 1995). Given the range of total lipid values spanned in this study, a comparison of total lipid levels in other studies with those in ours here should provide a good means to determine whether previous Iatroscan TLC–FID studies may indeed be biased low.

In summary, based on our comparison of our primary microquantity approach (microcolorimetric SPV) and a more traditional macroquantity approach, we feel that microquantity approaches offer clear advantages over macroquantity approaches when it comes to estimating total lipids of aquatic organisms, including fish. Most importantly, microquantity approaches can provide results comparable to those of macroquantity approaches with reduced processing time, solvent costs, and chemical waste. In addition, a reduced need for large tissue subsample volumes will in most cases eliminate the need to pool samples (per Ludsin and DeVries 1997), which has shown to be necessary when using macroquantity approaches (e.g., Miranda and Hubbard 1994). Even so, macroquantity approaches still

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may prove advantageous over microquantity approaches in some cases. In particular, we recommend the use of macroquantity approaches in situations in which high measurement precision, not just a reasonably accurate estimate of lipid levels, is required. In addition, although our microgravimetric and microcolorimetric SPV approaches appear suitable for organisms with both high and low lipids levels, our results suggest that the Iatroscan TLC–FID approach might only be suitable for quantifying total lipids in organisms with lower lipid levels than species such as walleye and lake whitefish.

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Marine survival of steelhead (*Oncorhynchus mykiss*) enhanced by a seasonally closed estuary

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Abstract: To investigate the role that estuaries play in the survival of steelhead, *Oncorhynchus mykiss*, we compared juvenile size at ocean entry with back-calculated measures of size at ocean entry for returning adults in Scott Creek, a representative California coastal stream. During the annual spring emigration, the largest smolts (>150 mm fork length (FL)) move directly to sea, while some smaller smolts remain in the estuary until sandbar formation creates a closed freshwater lagoon. High growth rates in the estuary throughout the summer result in a near doubling of fork length from the time of estuary entry (mean FL of spring migrants = 102.2 mm; mean FL of fall lagoon resident = 195.9 mm). Analysis of the scale morphology of returning adult steelhead indicates that there is strong size-dependent mortality at sea, with estuary-reared steelhead showing a large survival advantage, comprising between 87% and 95.5% (based on tag returns and scale analysis, respectively) of the returning adult population despite being between 8% and 48% of the annual downstream migrating population. Although the estuary forms less than 5% of the watershed area, it is critical nursery habitat, and steelhead population persistence in southern margin ecosystems may well depend upon healthy estuaries.

Résumé : Afin d'estimer le rôle joué par les estuaires dans la survie de la truite arc-en-ciel anadrome, *Oncorhynchus mykiss*, nous avons comparé la taille des jeunes au moment de leur arrivée en mer avec les mesures obtenues par rétrocalcul de la taille des adultes qui reviennent de l'océan au moment de leur propre entrée en mer dans Scott Creek, un cours d'eau côtier typique de Californie. Durant l'émigration annuelle du printemps, les saumoneaux les plus grands (>150 mm de longueur à la fourche (FL)) se rendent directement en mer, alors que certains des saumoneaux plus petits demeurent dans l'estuaire jusqu'à ce que la formation de barres crée une lagune fermée d'eau douce. De forts taux de croissance dans la lagune au cours de l'été produisent un doublement de la longueur à la fourche par rapport au moment de l'arrivée dans l'estuaire (FL moyenne des migrateurs du printemps = 102.2 mm; FL moyenne des résidents de la lagune à l'automne = 195.9 mm). L'analyse de la morphologie des écailles des truites arc-en-ciel anadromes qui reviennent de mer indique une forte mortalité reliée à la taille dans l'océan; les truites élevées dans l'estuaire possèdent un important avantage en ce qui concerne la survie; ils représentent entre 87 % et 95,5 % (d'après respectivement les retours d'étiquettes et l'analyse es écailles) de la population des adultes qui reviennent, même s'ils ne constituaient qu'entre 8 % et 48 % de la population annuelle en migration vers l'aval. Bien que l'estuaire forme moins de 5 % du réseau hydrographique, c'est un habitat de nourricerie important; il est bien possible que la persistance de la population de truites arc-en-ciel anadromes dans les écosystèmes situés à la bordure sud de l'aire de répartition dépende d'estuaires en bon état.

[Traduit par la Rédaction]

Introduction

Throughout much of their range, steelhead (*Oncorhynchus mykiss*) populations continue to decline despite federal Endangered Species Act (ESA) listings. This loss has been attributed to habitat degradation, water loss, and poor land management (Nehlsen et al. 1991; Busby et al. 1996). Still, the factors affecting the distribution and dynamics of steelhead populations are not well understood, and few studies have looked at juvenile rearing habitats and their effect on survival for these threatened populations. Because of their anadromous life cycle, salmonids inherently encounter several distinctly different habitats throughout their life history. The contributions of differential habitat use to the growth and survival of individuals may play key roles in their recruitment to adult populations and has been the focus of extensive study for some salmonids (Reimers 1973; Mitro and Zale 2002; Harvey et al. 2005) but largely ignored for steelhead.

During their seaward migration, salmon may enter estuarine habitats, which vary widely in their physical characteristics (Healey 1991). Estuaries are of particular interest because they have been found to be nurseries for many species of fishes and invertebrates (Sogard 1992; Yamashita et al. 2000; Le Pape et al. 2003), allowing juveniles using them to recruit disproportionately to the adult population compared with those from other habitats (Beck et al. 2001). Salmon use of estuarine habitats has been well documented for

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rivers from British Columbia to central California (Levy and Northcote 1982; McCabe et al. 1986; MacFarlane and Norton 2002). However, the time spent in an estuary and the benefits received from that habitat may vary widely among species and watersheds (Bottom et al. 2005). Some salmon move through estuaries in days, whereas others remain for months (Myers and Horton 1982; MacFarlane and Norton 2002; Miller and Sadro 2003). Increased survival of estuarine-reared individuals has rarely been documented (Reimers 1973), although rapid growth in the estuary has been documented for some species and populations (Healey 1979; Simenstad et al. 1982; Fisher and Pearcy 1990*a*).

Estuaries of coastal watersheds in the southwestern margin of North America commonly form ephemeral freshwater lagoons, seasonally closed to the coastal ocean. These lagoons are formed by low summer flow regimes that cannot displace ocean sand deposition at the mouths of the estuaries (Schwarz and Orme 2005). Eventual formation of a sandbar effectively blocks surface connectivity with the ocean and reduces the tidal influence on the system, creating a warmer, mostly freshwater, slow-moving body of relatively deep water. Lagoon conditions generally exist until the first winter pulse of freshwater increases streamflow and removes the sandbar, opening the lagoon to the ocean. The development of lagoon conditions and their effects on salmonids is not well understood. However, a recent study has shown a lagoon environment to be beneficial to the growth of juvenile steelhead in central California (Hayes et al. 2008). Because of their dynamic state and to avoid confusion, lagoon habitats will be referred to as the estuary for the remainder of this text, regardless of its connectivity to the ocean.

Ward et al. (1989) found a strong size-dependent ocean survival in British Columbia's Keogh River steelhead, with the largest smolts (i.e., juveniles migrating to sea) exhibiting a higher survival than smaller migrants. In addition, young steelhead have been observed in estuaries, where growing conditions may be favorable (Dawley et al. 1985; Quinones and Mulligan 2005); however, no research to date has successfully attributed survival of returning adults to estuarine rearing habitat. Higher ocean survival of estuary-reared steelhead would implicate the estuary as an important nursery habitat, especially given the small proportion that it represents of all freshwater habitats. In light of past and current declines of steelhead populations, it is necessary to make the link between individuals that recruit to the reproductive population and the factors that may have lead to their survival.

Here, we determined whether a coastal California estuary serves as a steelhead nursery habitat, producing a disproportionate contribution to adult populations than would be realized in its absence. To address both the importance and mechanism of the estuary as a nursery habitat, we investigated two key questions: (*i*) is there size-related differential marine survival in coastal California steelhead, and if so, (*ii*) do estuarine-reared juveniles experience higher marine survival and recruit to the spawning adult population in greater proportion than individuals that migrate directly to sea with minimal use of the estuary.

To determine the extent to which estuary residence influences the survival rate of steelhead at sea, we sampled both the juvenile and adult steelhead populations over several years (2002–2007) in a typical coastal watershed along the coast of central California. Initially, we estimated the strength of size-dependent ocean survival from a population of hatchery-reared juveniles and adults that were permanently marked (adipose fins removed) as smolts. We then estimated the abundance and size distribution of both the downstream migrating juveniles (both hatchery and wild-spawned smolts) and the subset of downstream migrants that recruited to the estuary and remained there throughout the summer and fall. Sampling of these groups effectively measured both of the dominant steelhead life-history types in Scott Creek: those individuals that move from the upper watershed to the ocean with minimal stay in the estuary and those that move from the upper watershed to the estuary for a period of months before beginning their ocean migration (Hayes et al. 2008). In addition, we used passive integrated transponder (PIT) tags to estimate juvenile-to-adult survival rates of a subset of individuals from both estuarine and upstream habitats. Finally, scale samples were taken from returning adults to identify the size at initial ocean entry and classify the juvenile rearing habitat (i.e., upstream or estuary) through scale morphology (Ward et al. 1989).

Materials and methods

Study site

Scott Creek is a small, coastal California, USA, watershed (~70 km^2) that empties into the Pacific Ocean 80 km south of the mouth of San Francisco Bay (Fig. 1). Large waterfalls form impassable barriers on each of the main tributaries, thereby restricting access by anadromous fish to just 23 km of stream. Flow in Scott Creek is highly variable among seasons and years. Peak winter flows can reach 28 m^3·s^{-1}, whereas summer and autumn flows can be reduced to 0.08 m^3·s^{-1} during an average year and the stream can run dry in the lower reaches during extreme droughts. Substrata throughout the watershed are mudstone and granite with particle sizes ranging from boulder to sand. The upper portion of the watershed is comprised of a high gradient stream dominated by a thick coastal redwood (*Sequoia sempervirens*) canopy. The lower gradient main stem of the creek has a low-density overstory cover composed of alders (*Alnus* sp.), with understory dominated by willows (*Salix* sp.). An area of low-lying stream near the ocean forms a small estuary, which is subject to periods of high salinity during large tidal and swell events. The estuary is surrounded by a bullrush (*Scirpus californicus*) marsh. Like many coastal California streams, a sandbar forms most summers, causing the estuary to become a freshwater lagoon with infrequent saltwater input from ocean swells and tides. These environmental features (i.e., the geomorphology and riparian vegetation) that characterize the Scott Creek watershed are representative of many coastal streams along central and northern California.

Fishes of Scott Creek include steelhead, coho salmon (*Oncorhynchus kisutch*), threespine stickleback (*Gasterosteus aculeatus*), prickly sculpin (*Cottus asper*), coastrange sculpin (*Cottus aleuticus*), and tidewater goby (*Eucyclogobius newberryi*). Juvenile starry flounder (*Platichthys stellatus*) and Pacific staghorn sculpin (*Leptocottus armatus*) are infrequently observed in the estuary.

A small conservation hatchery has operated continuously on Scott Creek since 1982, spawning a small number of

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Fig. 1. Map of the study site in Scott Creek watershed. Inset shows location in California on the US West Coast.

steelhead and coho salmon each spring from broodstock that are at least one generation removed from the hatchery (Hayes et al. 2004). Like many southern populations, steelhead in Scott Creek are listed as threatened by the ESA because of low population numbers, despite a relatively unaltered watershed.

Size-selective survival

To test for size-dependent survival of steelhead at sea, we compared the initial size distribution of a cohort of smolts as they migrated to sea with the juvenile size distribution of returning adults of that cohort when they returned to Scott Creek. For this, we used a population of marked (adipose fin permanently removed) hatchery-reared smolts released in the spring of 2003. Hatchery smolts enter the ocean soon after release; therefore we assume that the size distribution of hatchery fish prior to release closely resembled the distribution of smolts that migrate directly to sea (Hayes et al. 2004). We measured the fork length (FL; to the nearest millimetre) of 562 hatchery-raised smolts from the entire pool of 6880 individuals, 1 week prior to release from the hatchery. Size at ocean entry was determined for the adults of this hatchery-reared cohort when they returned in the winter and spring of 2004 and 2005 as 1- and 2-ocean-year fish using the scale method described below. The size distribution at release of hatchery smolts was compared with the back-calculated size at ocean entry of returning hatchery adults to determine whether processes of size-dependent ocean survival have an effect on the resulting adult population. The probability of steelhead straying to Scott Creek from other streams is extremely low (Shapovalov and Taft 1938), and all adults captured were assumed to be of Scott Creek origin for the purposes of our analyses. Hatchery adults that returned from the ocean in the winters and springs of 2004–2006 to spawn were sampled with a floating resistance panel weir, operated daily during the spawning run (Hayes et al. 2004). The weir had a trap box with a one-way door to capture steelhead moving upstream. Upon capture, each fish was identified as either of hatchery (n = 278) or of wild (n = 590) origin and was measured to the nearest 0.5 cm FL and weighed to the nearest 0.1 kg. A sample of 10–15 scales was taken from just above the lateral line on a diagonal between the posterior attachment of the dorsal fin and the anal fin (Maher and Larkin 1955).

To determine the size at ocean entry of surviving hatchery adult steelhead from the 2003 smolt class, we used the linear relationship between fish size and scale size (Ricker 1992) and the reliable changes in scale morphology that are associated with changes in growth and habitat (e.g., ocean entry checks, circuli spacing with growth). Scales were cleaned with deionized water and photographed using a microscope-mounted Nikon® digital camera (DXM1200 3840 × 3072 pixels). The most original, uniform scale from each fish was photographed and saved in the tag image file format (TIFF).

Once each scale had been photographed, OPTIMAS® software (version 6.5; Media Cybernetics, Inc., Silver Spring, Maryland) and a custom macro were used to analyze for total scale radius (SR, the distance from the focus to the edge of the scale), radius at ocean entry (OER, the distance from the focus to the ocean entry check), number and spacing of each freshwater and ocean circulus, and number of ocean annuli (Fig. 2). For ease of reading, all measurements were made 20° off the longest axis. A qualitative score for each analysis was noted with a rank of 1 to 3, with 1 being a very original (i.e., the scale has remained on the fish since scales were formed and has not been lost and regenerated), normally shaped scale with a high reading confidence. Only scales with a score of 1 or 2 were used in further analyses.

FL at the initial ocean entry check was back-calculated on scales from adult steelhead using a regression of FL on total scale radius (SR). A linear regression was created with original scales from 1251 juvenile and adult Scott Creek steelhead representing the complete range of sizes available. The relationship between SR and FL (Fig. 3) is described by

$$(1) \qquad \text{FL (mm)} = 0.1686\text{SR } (\mu\text{m}) + 34.872 \ (R^2 = 0.97)$$

An intercept of 34 mm is similar to other published values of FL at initial scale formation for *O. mykiss* (Snyder 1938; Kesner and Barnhart 1972; Hopelain 1998). There is some discussion in the literature as to the most appropriate method for back-calculation of size (Panfili and Troadec 2002); however, the Fraser–Lee method is widely used, and recent studies have empirically demonstrated its reliability in several fish species, including *O. mykiss* (Davies and Sloane 1986; Klumb et al. 1999). We employed the Fraser–Lee method (Fraser 1916; Lee 1920) in all back-calculations of fish length. Briefly, the Fraser–Lee method utilizes the relationship between fish length and scale radius at time of

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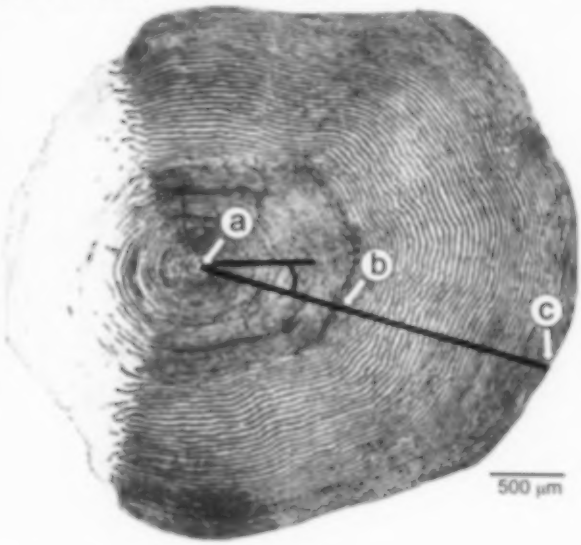
Fig. 2. Photograph of scale indicating (*a*) focus, (*b*) ocean entry radius (OER), and (*c*) scale radius (SR) and the 20° offset from the center axis used to make measurements.



capture to determine fish length at any radius along the scale when adjusted by the intercept of a fork length on scale radius regression for the species in question (Klumb et al. 1999).

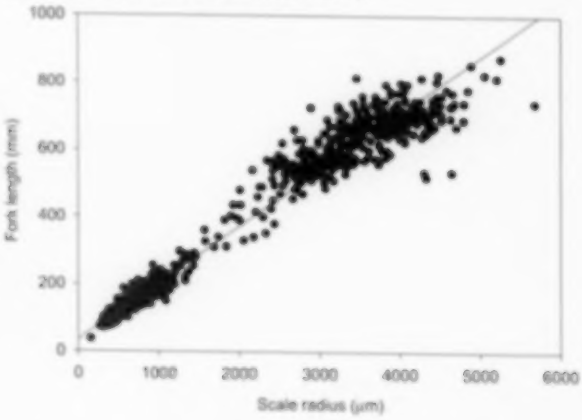
Size at ocean entry of estuarine-reared juveniles

To determine whether juvenile steelhead reared in strictly upstream habitats and the subset of those fish that remained in the estuary for an additional 6–9 months entered the ocean at different sizes and numbers, we trapped downstream migrants (smolts) each spring and summer and sampled the estuary population each fall (October–December). To determine the number and size of downstream migrants, we placed a fyke net across the stream approximately 50 m upstream of the estuary (Fig. 1). The fyke net (initial mesh size in 2002–2003 was 9.5 mm, switching to 6.4 mm in 2004 because of the small size of many migrants) was generally operated three days per week, January–July (2002–2005); however, storm events periodically prohibited the net from being operated. The number of downstream migrants was estimated by adjusting total fish captured (n = 1722) by capture efficiency (Hayes et al. 2004).

Captured steelhead were measured to the nearest millimetre FL, and mass was measured to the nearest 0.01 g. A sample of 10–15 scales was taken by running the blade of a pair of scissors in the posterior to anterior direction lightly along the side of the fish. Finally, each fish ≥65 mm FL was scanned for a PIT tag, using a handheld tag reader (Allflex USA, Dallas Fort Worth Airport, Texas). If no tag was found, then one would be injected (n = 1038) using a sterile 12-gauge needle.

The estuary habitat was sampled each fall (2002–2005) to determine the population size and the size distribution of juvenile steelhead that inhabited the estuary just prior to

Fig. 3. Relationship between fork length (FL) and scale radius (SR) based on scales from juvenile and adult steelhead (*Oncorhynchus mykiss*) collected throughout the watershed from 2002 to 2005 (n = 1250, FL = 0.1686 SR + 34.87, R^2 = 0.97).



ocean entry (referred to as "estuary juveniles"). The estuary habitat, defined as the area from the beach at the mouth of Scott Creek to approximately 800 m upstream (Fig. 1), was sampled using a 30 m × 2 m nylon beach seine. The entire estuary was seined as thoroughly as possible in 50 m sections, with the exception of the upper 200 m, where dense willow cover made seining impossible. All fish (n = 392) were placed into mesh containers in the estuary until all seining was complete so that fish were not collected twice. Estuary juveniles were assessed using the same protocol as trap-captured downstream migrants. However, the estimation of size at ocean entry required adjusting the size distribution of the last sampling each fall to account for growth occurring between the last sampling and sandbar breakage such that growth rates from the last sampling event (see Hayes et al. 2008) and the number of days between the last sampling and bar breakage were calculated and added to the final fall size distribution. Because of changes in sampling equipment and varying trap effectiveness among years, distributions could not be compared statistically among years, necessitating the grouping of juveniles into two distributions: spring downstream migrants (i.e., those fish that move from upstream habitats to estuarine and ocean habitats each spring) and fall estuary juveniles (i.e., the subset of spring downstream migrants that did not move to the ocean but remained in the estuary throughout the summer, as measured in the fall).

To explore those processes that might determine which fish remain in the estuary prior to migration to sea, we examined whether size at migration or timing of migration affected individuals present in the estuary after bar closure. To do this, we compared the size distribution of all downstream migrants with the size distribution at downstream migration of those PIT-tagged individuals that stayed in the estuary after sandbar closure. Data were organized into 15 mm FL bins from 85 to 145 mm, with all fish greater than 145 mm being grouped into the last bin of >145 mm. To determine what effect timing of downstream migration had in determining what individuals remained in the estuary by sandbar closure, we compared the trapping date for all

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fish tagged at the downstream migrant trap with the initial tagging date for those that remained in the estuary.

Effect of estuarine residence on recruitment to the adult population

We used three methods to determine whether estuary fish recruited disproportionately to the returning adult population. First, we calculated the size at ocean entry of returning adults and compared that distribution with the sizes at ocean entry of emigrating juveniles. Second, we classified returning adults to either upstream or estuary juvenile rearing habitat using maximum likelihood estimation and measures of scale morphology. Finally, we calculated return rates of adult steelhead that were PIT-tagged as juveniles at one of the two habitats to determine relative return rates for each habitat type.

We back-calculated the size at ocean entry of wild returning adult steelhead with the same scale measurement technique that was employed for returning hatchery fish. Scale samples were collected from 571 wild adults from spring of 2002 through spring of 2006. Although some 1-ocean-year fish were captured in 2006 and assigned to the 2005 ocean entry group, these samples were omitted from this analysis because of the potential bias of using only "early" returning fish to classify the entire 2004 cohort. In addition, scales with visible spawning checks (i.e., an area of degradation in the scale from previous stream entry) were removed to ensure that no fish was counted twice. After removals, 406 original, uniform scale samples that received a score of 2 or better during reading were used for the final analysis. Because of the difficulties in identifying freshwater annuli in adult scales, especially in estuary residents, returning adult steelhead were not assigned to a particular downstream migrant cohort for comparison. Instead, all returning adults were grouped together as one class, and their ocean entry FL was compared with the FL of fall estuary-reared fish and spring downstream migrants from all years. Unfortunately, trapping fish as they cross the sandbar to enter the ocean is not currently feasible, and size at ocean entry of fish that do not utilize the estuary must be inferred from the population that remains. This is difficult because of the potential, currently unknown, size-selective effects of predators during the migration. Fish not found in the estuary may have either gone to sea or been consumed by predators between the trap and the ocean. In this scenario, the most conservative comparison is to compare all spring downstream migrants and all fall estuary steelhead with the size distribution at ocean entry of the surviving adult population.

In addition to size, we used circuli spacing and spacing variance to distinguish between adults with significant juvenile estuarine rearing time and those that moved to sea without utilizing estuarine habitats. Circuli spacing (i.e., the width of the increment between adjacent circuli) in scales is correlated with growth in coho salmon (Fisher and Pearcy 1990*b*, 2005), sockeye salmon (*Oncorhynchus nerka*) (Fukuwaka and Kaeriyama 1997), and steelhead for which the origin of fish in mixed stocks of hatchery and wild steelhead has been determined and differences in circuli spacing attributed to the different growth regimes in the hatchery and the wild (Maher and Larkin 1955; Bernard and Myers 1996; Tattam et al. 2003). Upstream scales were collected throughout the year in the upper watershed, but because individuals only use estuary habitat after a prior stay in the upper watershed, estuary scales were taken in the late fall when the estuary growth signature has been maximized. To provide an indication of estuary-derived growth, we calculated the mean of log-transformed circuli spacing and variance for the last 18 circuli of juvenile fish of all size classes from the upper watershed ($n = 96$) and estuary ($n = 92$) for use in a discriminant function analysis (DFA). Although many combinations of circuli were tested in a stepwise fashion, the mean of the last 18 circuli (approximately 8–12 months of growth) was most effective at discriminating between prior habitat use, while simultaneously removing problems of non-independence in sampling. A maximum likelihood estimation (MLE) was then used to determine the composition of returning adult steelhead that reared in one of the two juvenile rearing habitats (upstream or estuary) based on the same parameters used in the juvenile DFA (mean log-transformed spacing of the last 18 circuli, variance of spacing). The maximum likelihood analysis results were produced with the program HISEA (Millar 1990).

To calculate the relative ocean survival of wild juvenile steelhead, we placed PIT tags in steelhead at both the downstream migrant trap and the estuary in the spring and summers of 2003–2005. Fish tagged at the downstream migrant trap and subsequently observed in the estuary after sandbar closure were removed from the trap population and added to the estuary population. We then scanned each returning adult for a PIT tag that could be attributed to deployment during one of these years and locations. Return data were used to calculate relative survival of individuals from each group.

Results

Size-selective survival

Hatchery smolts released in April of 2003 experienced strong size-selective mortality at sea (Fig. 4). Smolts measured just prior to release had a mean FL of 158 mm (standard deviation (SD) = 35). Original scales were obtained from hatchery fish returning as adults in the winter–spring of 2004 and 2005 as 1- and 2-ocean-year fish, respectively. Back-calculation of FL at ocean entry indicated that the surviving adult population had a mean FL at ocean entry of 181.2 mm (SD = 28.9), which was significantly larger upon ocean entry than the initial population of fish released from the hatchery ($t_{[592]} = 4.47$, $p < 0.001$).

Size at ocean entry of estuarine-reared juveniles

In 2003 and 2004, there were far fewer estuary fish than downstream migrants (48% and 8% of downstream migrant population, respectively) (Table 1). No population size was estimated for 2002 or 2005 because of the lack of early season samples due to excessive streamflow. Size distribution varied by sample site and year (Table 2), with the estuary residents being consistently larger than downstream migrants. The decrease in size after 2003 in both areas is likely attributable to switching to a smaller mesh net.

The size distribution of the estuary population upon bar breakage each winter varied by year; mean FL upon winter sandbar breakage was largest in 2003 at 213 mm (SD = 32)

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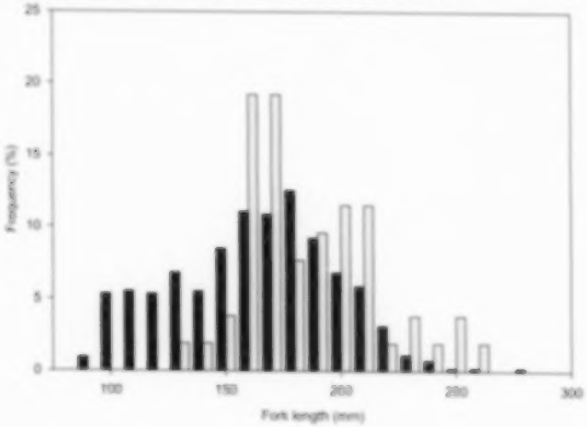
Fig. 4. Size distributions of juvenile hatchery-reared smolts (n = 542, solid bars) sampled immediately preceding release and the back-calculated size at ocean entry of surviving adults from the same cohort (n = 52, shaded bars). Bins are 10 mm.



and smallest in 2004 at 182 mm (SD = 26), but estuary fish from all years (2002–2005) were significantly larger than spring downstream migrating juveniles in the same years ($t_{[455.4]}$ = 45.76, $p < 0.001$).

Of the 298 fish that we measured and PIT-tagged at the downstream migrant trap in spring of 2003, 61 were recaptured in the estuary in the fall, well after sandbar formation. The FL at initial estuary entry was compared between the two groups to determine whether specific size classes were more likely to remain in the estuary (Fig. 5). The initial size of all downstream migrants was larger than the initial size of those individuals that remained in the estuary ($\chi^2_{(5,n=359)}$ = 15.36, p = 0.009), indicating that larger individuals moved directly to sea, whereas some of the smaller individuals remained to populate the estuary after sandbar formation. No fish with an initial estuary entry FL larger than 150 mm was observed after sandbar closure, indicating that those larger fish moved on to the ocean before sandbar formation.

Habitat-specific recruitment to the adult population

To determine whether juveniles that remained in the estuary contributed disproportionately to the subsequent recruitment of returning adults, we compared the size at ocean entry of juveniles from 2002–2005 that either migrated directly to sea or remained in the estuary with the back-calculated size at ocean entry of adults returning in the same years (Fig. 6). For all sampling years combined, FL at ocean entry differed significantly among the spring downstream migrants, fall estuary residents, and back-calculated returning adults (analysis of variance (ANOVA), $F_{[2,1802]}$ = 2192.9, $p < 0.001$). Post-hoc comparisons using the Tukey test indicated that there were significant differences among all three groups. The mean FL of spring emigrating smolts for all years was 102.2 mm (SD = 26, n = 1722), whereas the mean FL of fall estuary fish was 195.9 mm (SD = 28, n = 392), and ocean entry FL of returning adults was 208 mm (SD = 38, n = 406).

To provide another independent measure of the effect of juvenile rearing habitat on returning adult steelhead, we used measures of scale spacing as a proxy for juvenile growth, with larger spacing (i.e., increment widths between adjacent circuli) indicating faster growth. Mean spacing was significantly greater in juveniles that occupied the estuary than in those that did not ($t_{[180]}$ = 13.95, $p < 0.001$; Fig. 7). We were able to assign juveniles to their respective rearing habitat; DFA jackknifed classification, using circuli spacing and variance as predictors, indicated an 86% correct assignment (90% for upstream, 83% for estuary) to either habitat. Scales from adult fish (n = 406) were also analyzed to determine the mean spacing and variance for the last 18 circuli prior to ocean entry. Spacing was significantly wider than either the estuary or upstream individuals ($F_{[2,593]}$ = 151.8, $p < 0.001$). A MLE using mean log-transformed circuli spacing and variance of spacing as predictors indicated that 95.5% and 4.5% of returning adults had reared in the estuary and upstream waters, respectively.

Table 1. Proportion of downstream migrating juvenile steelhead residing in the estuary in 2003 and 2004.

	Year	
	2003	2004
Estimated total no. of wild downstream migrants	5272*	18 354
Estuary population ± SD	2540±479.4	1489±381.9
Downstream migrants utilizing the estuary (%)	48	8

*2003 is assumed to be an underestimate of the wild population due to the large net mesh size used in downstream migrant trapping. Data are from Hayes et al. (2004) and S.A. Hayes, unpublished data.

The strength of differential ocean survival of PIT-tagged estuary- and upstream-reared steelhead was estimated from adults captured in the weir from the winter of 2004 through the spring of 2007. To date, 52 wild steelhead have returned from the ocean carrying PIT tags implanted either at the downstream migrant trap or in the estuary. All fish were tagged as juveniles from 2003 through 2005. In those years, 1260 juveniles were tagged at the downstream migrant trap; however, 169 of these were later observed in the estuary and were removed from the trap totals, leaving a final count of 1091 fish tagged at the downstream migrant trap. A total of 1053 fish were tagged or observed in the estuary after sandbar formation. Forty-five of 52 returning adults were from the estuary juvenile pool, whereas only seven were from the downstream migrant trap. After adjusting for juvenile tagging effort, captures to date indicate that approximately 87% of returning adult steelhead are products of lagoon rearing, whereas 13% were from exclusively upstream habitats.

Discussion

This study provides compelling evidence for the importance of estuarine habitat as a nursery to coastal California steelhead populations. Although estuaries have been shown to function as nurseries for other fish species (Epifanio et al. 2003; Brown 2006), their effect on the relative survival of steelhead was previously unknown. A strong size-selective ocean survival coupled with a large dichotomy in sizes between estuary- and upstream-reared smolts has led to a survival advantage for the larger estuary-reared individuals.

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Table 2. Comparison of the mean size (fork length (FL)) of downstream migrant juvenile steelhead sampled in spring with that of estuary resident juvenile steelhead sampled in both the late spring and fall of each year.

		Downstream migrants		Estuary residents			
				Late spring		Fall	
Year	Trapping dates	*n*	Mean FL (mm) ± SD	*n*	Mean FL (mm) ± SD	*n*	Mean FL (mm) ± SD
2002	April–July	398	110.2±25	19	112.9±12	44	196.2±21
2003	January–July	448	110.0±29	17	108.6±13	81	213.6±32
2004	January–July	665	92.6±24	51	112.1±39	148	182.5±26
2005	March–July	211	96.0±25	102	91.7±38	119	191.1±33
All years		1722	102.2±26	189	106.3±26	392	195.9±28

Fig. 5. Comparison of size distributions of spring downstream migrants passive integrated transponder (PIT)-tagged prior to estuary entry (n = 298, solid bars) and the size at initial estuary entry of tagged fish recaptured in the estuary after sandbar closure (n = 61, shaded bars). Bins are 10 mm.

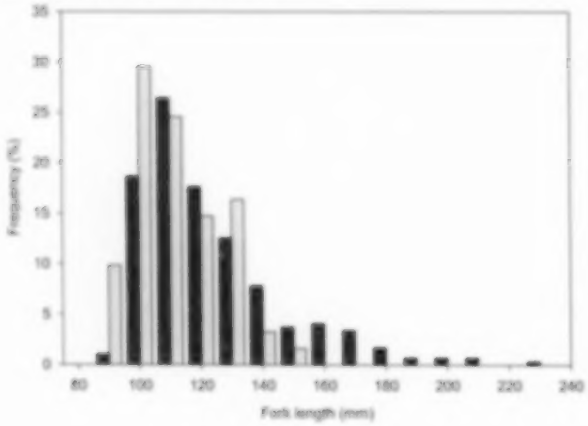
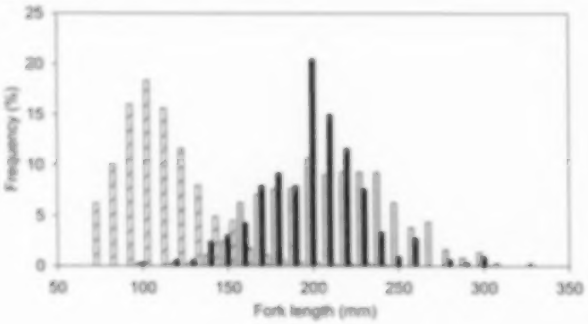


Fig. 6. Summed size distribution of all downstream migrants in 2002–2004 (n = 1300, hatched bars), late fall estuary residents in 2002–2005 (n = 327, solid bars), and back-calculated size at ocean entry of adults returning in 2002–2005 (n = 364, shaded bars). Bins are 10 mm.



These patterns are driven by the difference in growth rates between productive estuary waters and the relatively oligotrophic upstream habitat (Hayes et al. 2008). As most of the estuaries along the California coast have suffered anthropogenic impacts (e.g., channel modifications for buildings, bridges, agriculture, and water diversions), this is likely to have played a major role in steelhead declines in California in the last century (Busby et al. 1996).

Although evidence of size-selective survival in fishes is not new (Sogard 1997), the strength of size-selective survival combined with an extreme dichotomy in sizes of ocean entry between the two general rearing habitats (upstream and estuary) could lead to size-selective survival being the largest determinant in driving which individuals ultimately contribute to the adult population. Back-calculated size at ocean entry for 2003 hatchery juveniles returning as adults in 2004 and 2005 indicated that small smolts (≤150 mm FL) were underrepresented in the returning adult population, and larger smolts (>200 mm) were overrepresented. These data support the size-biased survival proposed by Ward et al. (1989) for a northern stock of steelhead. It has been shown that hatchery-reared salmon may experience lower overall survival in the marine environment (Jonsson et al. 2003); however, the processes shaping the size distribution of surviving fish (i.e., predation, foraging success) should act similarly on both hatchery and wild populations. This would suggest that wild Scott Creek smolts also experience a strong size-biased survival, with the largest fish returning in much greater numbers than those moving to sea at a smaller size. Indeed, our observations of few fish less than 150 mm FL at ocean entry (back-calculated) returning as adults, despite the vast majority of downstream migrating fish in the <150 mm FL size class, further suggests the size-selective survival of wild fish. This apparent 150 mm FL survival threshold is in agreement with a size-selective survival estimate for a northern steelhead population in which survival of smolts below 150 mm was near zero (Ward and Slaney 1988), suggesting that common ocean selection patterns exist across steelhead populations. The basis for reduced survival of the smaller fish remains unclear, but it is likely that the smaller individuals are more susceptible to starvation (Simpkins et al. 2004), predation, and disease (West and Larkin 1987; Becker et al. 2005).

Spring downstream migrants enter the Scott Creek estuary at a relatively small size compared with smolting steelhead in more northern populations (Ward and Slaney 1988; Ward et al. 1989). This is consistent with the relatively low growth rates observed in upstream habitats of Scott Creek (Hayes et al. 2008) and the large numbers of small downstream migrants observed by Shapovalov and Taft (1954) in nearby and physiographically similar Waddell Creek. Although the estimated number and mean size of down-

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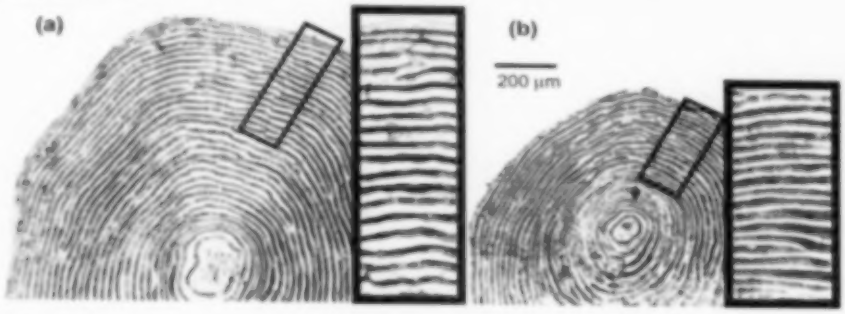
Fig. 7. Comparison of representative scales from juvenile steelhead from (*a*) estuarine habitat (170 mm fork length (FL), mean spacing of last 18 circuli = 27.9 μm) and (*b*) upstream habitat (158 mm FL, mean spacing of last 18 circuli = 2.5 μm). Inset for each scale is an enlargement of the last 18 circuli used in the discriminant function analysis (DFA) and illustrates the difference in mean spacing among fish from estuarine and upstream habitats.



stream migrants differed annually in this study (due to a change in net mesh size), these differences tended to overestimate size at downstream migration. The results still conservatively indicate that the vast majority of Scott Creek steelhead move downstream at a size that is detrimental to marine survival. Many studies have discounted small migrants that do not possess typical smolt physical characteristics (e.g., low condition factor, silver coloration, black tail) as migratory parr that do not enter the marine environment. In southern populations, however, many of these individuals have elevated gill Na^+/K^+-ATPase activity, a common indicator of seawater readiness (C.V. Hanson, unpublished data), and appear to enter the ocean at a small size.

The estuary population of steelhead is comprised of juveniles that emigrated from the upper watershed in the spring and summer. Downstream migrants greater than 150 mm FL move through the estuary and are not observed again as juveniles, suggesting they are large enough to compete successfully in the ocean. Large fish moving directly to sea may also help to explain why the hatchery fish, generally released at double the FL of wild downstream migrants, are rarely observed in the estuary. Most steelhead in Scott Creek appear to be migrating at smaller sizes specifically to benefit from greater estuary growth potential, thus allowing them to enter the ocean at a favorable size and younger age than those reared exclusively in upstream waters (Hayes et al. 2008). The estuary population each fall varied between 8% and 48% of the estimated total number of downstream migrants (in 2004 and 2003, respectively); however, the 48% estuary utilization in 2003 is probably a substantial overestimate (again due to large mesh size passing smaller fish, effectively underestimating the number of downstream migrants). Timing of sandbar formation does appear to impact the overall number of downstream migrants that will reside there. In years when high flow prevents early season sandbar formation, productive deep water is not found until the late summer and may harbor fewer fish. On the other hand, early sandbar formation during low flow years leads to productive habitat being available during peak downstream migration and may cause more fish to remain in the estuary throughout the summer. These considerations suggest that future year-class strength may be due, in part, to the timing of sandbar formation.

It is possible that estuary mortality is low in Scott Creek, which would favor juveniles using the estuary before entering the ocean. Unlike larger estuaries, no marine mammals have been observed in the Scott Creek estuary, and in its closed state, aquatic predators are prevented from entering from the ocean. Prickly sculpin have infrequently been observed feeding on smaller steelhead in the upper watershed; however, most steelhead entering the estuary are probably large enough to avoid predation by prickly sculpin. Avian predators are an important source of estuarine mortality for some salmonids, particularly steelhead in the Columbia River, with birds consuming greater than 10% of the steelhead previously detected moving into the estuary (Ryan et al. 2003). Avian predators (e.g., cormorants (*Phalacrocorax auritus*), brown pelicans (*Pelecanus occidentalis*), gulls (Laridae)) are often present in the Scott Creek estuary but their relative impact is unclear as the deeper estuarine water may provide a refuge from the avian predators (e.g., mergansers (*Mergus* sp.), kingfishers (*Ceryle alcyon*), great blue herons (*Ardea herodia*)) that readily feed on steelhead in the shallower upstream waters. Further study is required to determine what impact predation by seabirds has on the distribution and density of steelhead in the estuary. It is certainly possible, though, that steelhead evolved to utilize the Scott Creek estuary specifically because of the excellent growth opportunity and the relatively low predation pressure compared with marine environments. However, there are likely risks associated with estuarine rearing, otherwise the larger downstream migrants, which tend to pass through, might be expected to stay and increase their chances of marine survival associated with additional growth. These risks include intolerable summer water temperatures, loss of habitat from drought, the intrusion of anoxic saltwater layers and sediments, and predation, any of which may lead to high estuary mortality. In addition, strong density-dependent growth rates could cause fish to enter the ocean upon sandbar breakage at a smaller than optimal size in years of high lagoon steelhead densities (Hayes et al. 2008). It is also unclear when estuary-reared juveniles move to sea. Juveniles moving to sea immediately upon estuary sandbar breakage would experience further selection pressure from ocean conditions, which may be poor in the winter when breakage often occurs. Therefore, juvenile steelhead condition (e.g., size, energy stores) coupled with timing of ocean entry may be a major factor driving demography of the adult population (Limburg 2001).

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As a whole, small coastal estuaries in central and southern California appear to function much differently than larger estuaries (e.g., Columbia River, San Francisco Bay). Many of the larger estuaries have extensive populations of large piscivorous fish (e.g., flatfish (Pleuronectiformes), cutthroat trout (*Oncorhynchus clarki*), striped bass (*Morone saxatilis*)), and potentially vast communities of competitors (e.g., other salmonids (*Oncorhynchus* sp.), perch (Percidae), shad (*Alosa sapidissima*), smelt (Osmeridae), sole (Soleidae)) where extended residence in these areas may not offer the same advantages that smaller estuaries with few other fish species provide.

Back-calculation of size at ocean entry from the morphological characteristics of scales of returning adults indicates that surviving adults were quite large as juveniles upon ocean entry. In fact, the vast majority of survivors were so large at ocean entry that the upstream waters alone could not have produced them, as indicated by the size of downstream migrants. Size-selective survival in both wild and hatchery fish indicates that small fish are less likely to survive in the marine environment and estuary-reared juveniles constitute most of the returning adult population. Using MLE, we determined that over 95% of adults returning from 2002–2006 were at least partially reared in the estuary as juveniles. In addition to habitat assignment through MLE, the comparison of spring smolt versus fall estuary-reared size distributions to back-calculated size at ocean entry of adults gives an independent measure of habitat use that emphasizes the estuary as having been used by most surviving adult steelhead as juveniles. The estuary fills the role of an important nursery in this system by its disproportionate contribution to the adult population that returns to spawn. Unlike some nurseries, however, estuarine rearing requires the migration of fish from the upper watershed and cannot function as the sole rearing habitat for juvenile steelhead. Loose gravel with consistent flow, found only in the upper watershed, is required to incubate steelhead eggs. In addition, the benefits of the estuary habitat observed in the spring, summer, and fall months may not be present in the winter when large flows scour the estuary, leaving little refuge, and potentially disturb anoxic sediments.

The vast majority of the PIT-tagged adult steelhead returning from the ocean were originally tagged or observed in the estuary (87%). Returns of trap tagged fish were poor (13%). Although it is possible that some trap fish moved to the estuary and remained unobserved throughout the summer, adult returns of trap fish should reflect this with higher return rates than we have observed. We feel that these data are in strong agreement with the scale morphology analysis and lend further support to our stance that the estuary is functioning a nursery for young steelhead.

Our study has shown that estuarine rearing is a significant contributor to steelhead population persistence. The results of this study indicate there is size-selective ocean survival of central California coastal steelhead. Further, our results strongly suggest that the estuary is an important nursery habitat for producing large juvenile steelhead with increased ocean survival. Heavy alteration of the Scott Creek estuary from highway development in the 1930s has left the estuary substantially smaller than its historic size and may have drastically altered the hydrodynamics of the system. Estuarine waters in Scott Creek now comprise less than 5% of the habitat available to steelhead, yet the vast majority of the adult population is a product of that environment. Anecdotal estimates of run sizes from Scott Creek prior to highway development suggest spawning adult numbers two to three times larger than those observed today (e.g., California Department of Fish and Game 1910). More work is needed to determine what strategies steelhead utilize in watersheds without estuaries to achieve a size large enough to survive at sea without the additional growth that these habitats afford. Finally, the strength of size-selective mortality in the ocean appears to be sufficient that the very small size at ocean entry for many Scott Creek smolts would eliminate them from the population. Hayes et al. (2008) observed density-dependant growth rates among years for steelhead in the Scott Creek estuary, indicating that there may be a spatial limit on how many fish can recruit to the estuary in some years. Thus, more work is also needed to determine the consequence of increasing the size and duration of estuarine habitat on the size and dynamics of steelhead populations and how density-dependent processes influence these relationships.

These results indicate that coastal estuaries, which are often the most impacted parts of a watershed, may be more important to steelhead persistence in the southern portion of their range than previously thought and that their degradation could have drastic implications for imperiled steelhead populations. Indeed, future efforts to protect and restore steelhead populations in small watersheds should prioritize healthy and sizeable estuarine habitat as an effective method of returning steelhead to their historic numbers. In addition, northern stocks may come to rely on the added resiliency that healthy estuaries provide, as perturbations from habitat alteration or climate change alter the function of traditional rearing habitats.

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A modelling study of Norway lobster (*Nephrops norvegicus*) larval dispersal in southern Portugal: predictions of larval wastage and self-recruitment in the Algarve stock

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Abstract: A set of simulations using a validated and realistic parameterization of a numerical model was conducted for the south and southwest Portuguese regions as an attempt to understand larval dispersal patterns in the Norway lobster (*Nephrops norvegicus*). Larvae were introduced in the model as Lagrangian particles with five different behavioural scenarios concerning their ability to migrate vertically. Growth rate was temperature dependent and the larvae were tracked individually. The end point of the simulations was the position of the larvae when they reached competency at age 1. Age 1.25 was also considered to simulate a possible delay in settling due to lack of an appropriate substrate. The results showed that the majority of the larvae reached age 1 near the hatching area along the southern shelf, while low exchange of larvae between the south and the west coasts was observed, especially for behavioural scenarios where larvae remained in relatively shallow waters. Scenarios where larvae performed diurnal vertical migration and delayed settlement until age 1.25 indicated a tendency for westward motion because of interactions with the Mediterranean undercurrent. Self-recruitment to the Algarve stock was estimated at 0.2% to 0.5%, raising the concern that this stock may be experiencing recruitment limitation.

Résumé : Nous avons réalisé une série de simulations à l'aide d'un modèle numérique doté de paramètres validés et réalistes dans les régions portugaises du sud et du sud-ouest pour tenter de comprendre les patrons de dispersion des larves chez le homard de Norvège (*Nephrops norvegicus*). Nous avons introduit les larves dans le modèle comme des particules lagrangiennes possédant différents scénarios comportementaux relatifs à leur capacité de migration verticale. Le taux de croissance est dépendant de la température et les larves ont pu être suivies individuellement. Le terme final des simulations est la position des larves au moment où elles peuvent vivre indépendamment à l'âge 1. Nous avons aussi examiné l'âge 1.25 pour simuler la possibilité de délais dans l'établissement dus à la pénurie de substrats appropriés. Les résultats montrent que la majorité des larves atteignent l'âge 1 près du lieu d'éclosion le long de la plate-forme sud; on peut cependant observer un faible échange de larves entre les côtes sud et ouest, particulièrement dans les scénarios comportementaux dans lesquels les larves demeurent en eaux relativement peu profondes. Les scénarios dans lesquels les larves font des migrations verticales diurnes et retardent leur établissement jusqu'à l'âge 1.25 montrent une tendance vers un déplacement vers l'ouest à cause des interactions avec le courant sous-marin de la Méditerranée. Nous estimons l'auto-recrutement du stock de l'Algarve à 0,2 % à 0,5 %, ce qui soulève des inquiétudes sur la possibilité de limitation du recrutement dans ce stock.

[Traduit par la Rédaction]

Introduction

The Norway lobster (*Nephrops norvegicus*) is a burrowing decapod crustacean with an extended distribution in the palearctic Atlantic and Mediterranean regions. According to the International Council for the Exploration of the Sea (ICES 2006), this species is commercially exploited throughout its geographic area, with landings around 600 t per year in the ICES area IXa (West Galicia, Portugal, and Gulf of Cadiz). The economic importance of the species has promoted considerable interest in several aspects of its biology, such as growth, reproduction, and spatial structure of

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the populations (e.g., Farmer 1974; Maynou et al. 1998; Tuck et al. 2000). The species is one of the most valuable demersal shellfish resources in Portugal, where it is targeted, along with a mix of other species, by the demersal trawl fleet. Until the late 1980s, *Nephrops* was the main species captured by the trawl fleet, but catches have decreased considerably during the 1990s to minimum values in 1996 and 1997 (ICES 2006). This situation led to the implementation and enforcement of a recovery plan applied to the Iberian Atlantic stocks since 2006. While the observed catch decrease is largely caused by the fishery pressure, there is the concern that some of the variability could be due to environmental effects, particularly during the larval phase.

It is commonly accepted that oceanic currents play a major role in the retention and (or) dispersal of *Nephrops* larvae from hatching areas and in the recruitment back to settlement sites (White et al. 1988; Hill 1990; Bailey et al. 1995). In the Irish Sea, where most larvae occur between surface waters and 50 m depth (Harding and Nichols 1987; Lindley et al. 1994), larvae can be transported away from hatching areas by the density-driven circulation (White et al. 1988). Diel vertical migration (DVM) is a common behavioural pattern in decapod larvae that influences dispersal distances and pathways (Queiroga and Blanton 2004), and in the shallow North and Irish seas *Nephrops* zoeae have been reported to perform this type of migration, occurring close to the surface at night (Hillis 1974; Lindley et al. 1994). However, nothing is known about the vertical distribution of this species' larvae along the Portuguese and Mediterranean coasts, where *Nephrops* lives much deeper. Interest on the larval ecology of *Nephrops* has also been raised by several studies on genetic geographic structure (Maltagliati et al. 1998; Stamatis et al. 2004, 2006), which consistently showed small but statistically significant genetic divergence at the regional scale (10s to 100s of kilometres) but a lack of isolation-by-distance differentiation, as well as phylogenetic trees without geographical meaning, across the distributional range of the species (1000s of kilometres). Given that adults are sedentary, most of the dispersal should be accomplished during the larval phase. The lack of geographic structure at the large scale has been interpreted as resulting from post-Pleistocenic range expansion shaping the current genetic structure of *Nephrops* polulations and not by exchange of larvae among such distant regions as the North and the Aegean seas (Stamatis et al. 2004). However, the question remains open on the importance of larval exchange on demographic and genetic connectivity between populations at scales of 100s of kilometres.

Nephrops norvegicus adults are commonly found in depths ranging from 90 to 800 m along the Portuguese shelf and slope areas, on bottoms composed of sticky mud (Figueiredo and Thomas 1967). The distribution is patchy, with the largest area of high abundance located off the south coast of Portugal, in the plateau areas surrounding the Diogo Cão deep (centred at 36.67°N, 7.67°W). Egg spawning in Portugal occurs in August and September, and the ovigerous period lasts about 28 weeks. Hatching of larvae takes place from January to April, when first zoeae have been found in coastal waters (dos Santos and Peliz 2005). The larval phase includes three zoeal stages whose duration are temperature dependent. Based on laboratory experiments (Figueiredo and Vilela 1972), the estimates of the time that the zoeae take to reach metamorphosis to the megalopa stage range from 15 days (at 17 °C) to 45 days (at 8 °C). Metamorphosis presumably takes place in the plankton, after which the megalopa settles on the bottom.

Despite *Nephrops* adults being one of the most abundant crustacean species inhabiting slope areas, larvae have been comparatively rare in plankton samples collected during 41 oceanographic surveys conducted over a period of 15 years off the Portuguese coast (dos Santos and Peliz 2005). Various factors may contribute to the scarcity of the larvae, from a relatively low fecundity compared with other decapod species to a depressed density of the stocks caused by the fishery. An important factor may also be the slope habitat occupied by the species and the exposure of the Portuguese coast to the open ocean. *Nephrops* larvae are exposed to a huge volume of ocean following hatching, and the interaction between ocean currents in an unbounded ocean with larval behaviour may be a key issue for dispersal, recruitment back to parental populations, and connectivity among high-abundance areas.

The concerns of the present study were to determine to which degree *Nephrops* larvae produced off the south coast of Portugal are transported away from hatching areas and if such transport represents a possible contribution to neighbouring settlement areas. Given the rarity of the larvae in the plankton and the absence of adequate data on their spatial distribution, we used a modelling approach. To this end, a numerical model of the circulation was developed for the south coast of Portugal, to which an individual-based model (IBM) was coupled. The IBM allowed us to simulate the effect of temperature on larvae development rate, as well as incorporate several larval behaviour scenarios.

Models and methods

Ocean model

The simulations were conducted using a free-surface, terrain-following, primitive equation hydrostatic model configurable for fully realistic regional applications. We have used a version of the Regional Ocean Modeling System, ROMS (Shchepetkin and McWilliams 2003, 2005; Wilkin et al. 2005), with embedded nesting capabilities, ROMS-AGRIF (adaptive grid refinement in fortran; Penven et al. 2006). ROMS-AGRIF enables the use of online and offline nesting, thus permitting the regional applications to be built based on basin- and local-scale configurations and to cover a wide range of time and space scales. Examples of ROMS-AGRIF application to western Iberia are reported in Peliz et al. (2007*a*, 2007*b*) and Teles-Machado et al. (2007).

The present configuration was developed based upon the numerical study of Peliz et al. (2007*a*). A summarized description is given here, and for full details about the model configuration, the reader is referred to that paper.

For a realistic simulation of the Gulf of Cadiz and the Southwest Iberian margin, it was necessary to include local and small-scale aspects like the Gibraltar Strait exchange (Mediterranean inflow and outflow) and the wind forcing with sufficient detail to cope with the strong variability in this region. On the other hand, the remote circulation influencing the western limit of the study region (Fig. 1*b*) is as-

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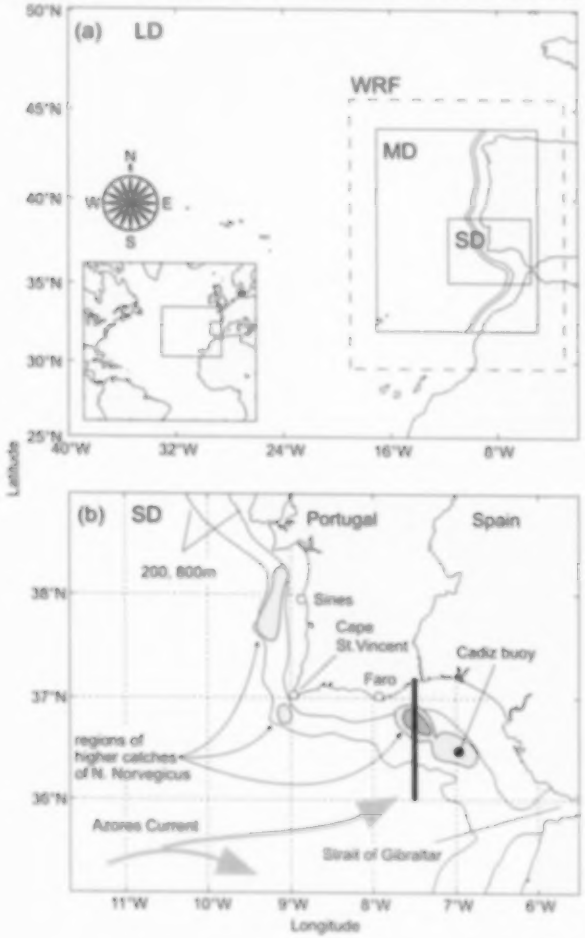
Fig. 1. Nested grid configuration used in this study. The bathymetric contours represent the depths of 500 and 1000 m (*a*) and 200 and 800 m (*b*). The spots between the isobath 200 and 800 at (*b*) are the regions with higher catches of *Nephrops norvegicus* adults. The vertical line at longitude 7.5 W indicates the location of the vertical slice shown in Fig. 8. SD, MD, and LD are the small, medium, and large ocean model domains, respectively, and WRF is the atmospheric model domain (i.e., weather research and forecast).



sociated with the Azores Current, which needs to be represented in the model. To resolve the large and small scales, three grids were used (Fig. 1*a*). First, a large domain grid (LD), with resolution around 16 km, between 2 W and 40 W and 25 N to 50 N was used to provide initial fields and lateral boundary conditions to an intermediate domain (MD) with ~9 km resolution (see Fig. 1). In this first step, the communication between LD and MD was done through offline nesting. Secondly, the MD provides information at the model time step (online nesting) to the small ~3 km resolution domain (SD), which constitutes the target domain for the dispersion study, and whose main features are described below.

The LD domain was initialized from rest using monthly temperature and salinity climatological fields (Levitus and Boyer 1994; Levitus et al. 1994) and was forced using monthly surface fluxes from Comprehensive Ocean–Atmosphere Data Set, COADS (da Silva et al. 1994). Along the lateral boundary conditions (south, west, and north), the same climatologies were used for temperature and salinity. Cross-boundary geostrophic velocity plus surface Ekman flow, computed from climatological density and climatological winds, respectively, were also provided on a monthly basis.

This LD configuration was run for 8 years, and equilibrium solutions are reached after 3 years. Although no interannual variability is allowed in the atmosphere nor in the lateral boundary forcing, small differences are observed among the 8 model years. The results from model year 4 LD were used for the initial fields and boundary conditions to the high-resolution-nested simulations (MD with embedded SD). The choice of year 4 for the base experiments is due to the fact that the solution is already in equilibrium, and the temperature and salinity characteristics at the levels of the Mediterranean water mass did not diverge substantially from climatological values, since its representation in the LD configuration is not explicit (a nudging term is used; Peliz et al. (2007*a*)).

In the case of the nested experiments, a realistic Mediterranean undercurrent (MU) was imposed by a boundary inflow and outflow condition at the Strait of Gibraltar (see Peliz et al. 2007*a* for details). This condition induces a slope circulation system that includes the MU and other surface currents (Peliz et al. 2007*a*). The nested experiments were forced with realistic atmospheric model data for the winter period of 2005–2006 (see below), and the ocean–atmospheric fluxes were calculated internally through a bulk flux formulation. The simulations starting date was 1 December 2005, and the experiments were conducted for 4 months. The winter 2005–2006 was characterized by the absence of abnormal atmospheric or oceanographic phenomena and may be considered as a typical winter representative of the present climatic conditions. On the other hand, the interannual variability of larval dispersal is beyond the scope of this study.

To assess the role of ocean internal variability (fluctuations in the current systems not driven by the atmosphere), the nested experiments were also initialized and controlled along the open boundaries with years 3 and 5 LD runs. However, the changes in the slope currents from the different years are small, because the system is largely conditioned by the flow structures associated with the Mediterranean inflow and outflow, which tends to be very stable in nature, and consequently it is kept fixed in the model (see Peliz et al. 2007*a* and references therein). Moreover, in the perspective of the main patterns of larval dispersal, no major differences were observed between the different years, and these experiments are not considered in the analysis below.

Atmospheric model

The global atmospheric reanalysis available for realistic ocean modelling are usually of too low resolution (in the order of 100 km), and the local scale shelf and slope circulation require winds with local detail (Teles-Machado et al. 2007). For that reason, the weather research and forecast

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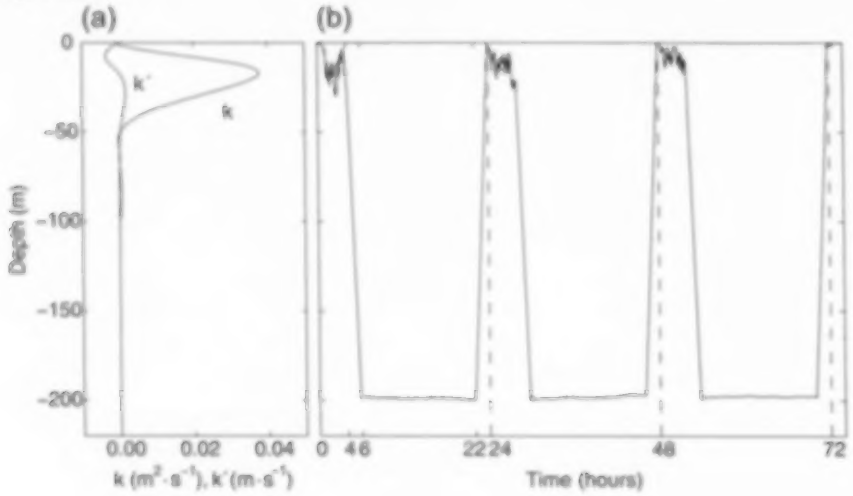
Fig. 2. (*a*) Vertical profile of turbulent diffusivity k and its vertical derivative k', leading to a vertical mixing of the particles through a random process. (*b*) Example of vertical location of a particle in the DVM200 scenario (diel vertical migration between the surface and a depth of 200 m) during 3 days.



(WRF) atmospheric model was used to dynamically downscale atmospheric reanalysis for the study period. WRF is a widely used research and forecast system (Skamarock et al. 2005), and an application of WRF simulations with ROMS is reported in Teles-Machado et al. (2007). A regional configuration of WRF (see WRF domain in Fig. 1*a*) was set up for the study period from 1 December 2005 to 31 March 2006. The WRF simulations were initialized and forced along the open boundaries with the National Centre for Environment and Prediction reanalysis data (Kalnay et al. 1996). WRF version 2.0 was used with a grid of 15 km and 60 vertical levels. Surface winds, humidity, pressure, temperature, and radiative data were generated and passed to the ocean model to calculate the air sea fluxes. For more details, the reader may refer to Teles-Machado et al. (2007) and Peliz et al. (2007*a*).

IBM

An IBM was coupled to the ROMS-AGRIF model to simulate hatching, development, and behaviour of the larvae. Coupling of the IBM and ROMS-AGRIF was made using a Lagrangian module (Capet et al. 2004; Peliz et al. 2007*b*). At each time step, particles introduced into the model to simulate larvae were advected by the three-dimensional velocity field resulting from the ocean model, with a high-order predictor–corrector time step algorithm. In addition to the advection generated by the model velocities, particle movements included random velocities in the horizontal (controlled by the explicit Lagrangian diffusion) and vertical (controlled by the vertical turbulent diffusivity) direction, which were used to parameterize unresolved turbulent processes. The reader is referred to Peliz et al. (2007*b*) and references therein for a detailed description of the Lagrangian module.

As well as to the advective and diffusive components described above, several behavioural scenarios were imposed on the particles regarding their initial distribution in the water column and their passive or active vertical swimming in an attempt to bracket the uncertainty about the behaviour of *N. norvegicus* larvae. In some of the experiments, a DVM scheme was explicitly introduced to simulate the vertical motion of larvae within the water column depending of the hour of the day. The DVM scheme consisted of forcing the larvae to drift at a deep level (described below) between 0600 and 2200 and at the surface between 0000 and 0400 every day, while during the remaining periods (0400 to 0600 and 2200 to 2400), the larvae migrated vertically from surface to a determined deep level and from that deep level to the surface, respectively (Fig. 2*b*). The scenarios were labelled and defined as follows: U5, passive larvae (no DVM) released uniformly within a 5 m thick surface layer; U50, passive larvae released uniformly between the surface and a depth of 50 m; U200, passive larvae released uniformly between the surface and a depth of 200 m; DVM200, larvae undergoing diel vertical migration between the surface and a depth of 200 m (Fig. 2*b*); DVM400, larvae undergoing diel vertical migration between the surface and a depth of 400 m. We did not include DVM scenarios within the 50 m surface layer. This option was taken because the mixed surface layer is typically deeper than 60 m during winter and because turbulent diffusivity would act as a mixing mechanism overriding the effect of vertical migration on the distribution of the larvae (see Fig. 2*a* for a typical vertical profile of diffusivity (k) and its vertical derivative (k') from which the vertical random displacements are calculated; see Peliz et al. 2007*b*, eq. 5; this effect on depth time series is exemplified in Fig. 2*b*). Because the simulations time started at 0000, larvae from DVM scenarios were released at the surface (DVM larvae are near the surface between 0000 and 0400). The deeper limits of the different scenarios were selected based on whether the larvae prefer surface layers during their development (5 m), considering the chlorophyll maximum layer (50 m) (Moita 2001), the euphotic layer (200 m), and the Mediterranean current upper layer limit

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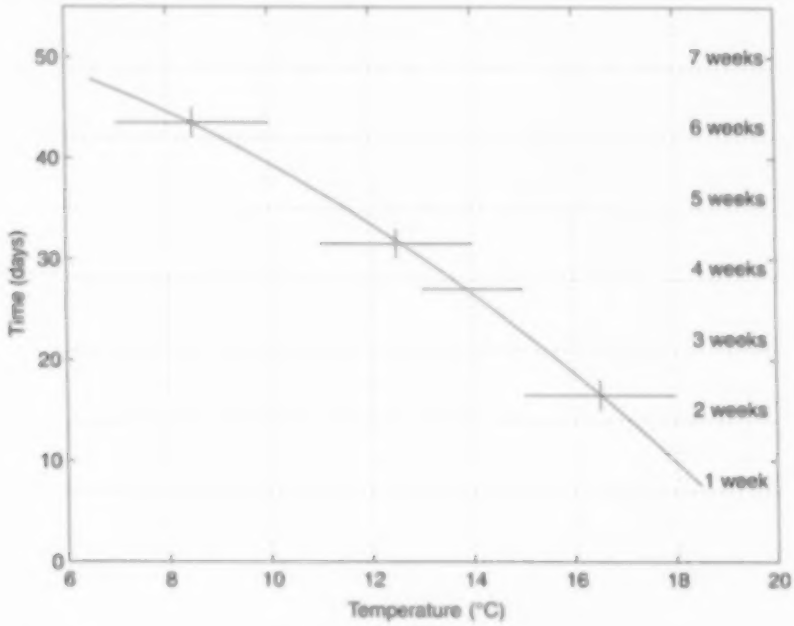
Fig. 3. Temperature-dependent development of laboratory-reared *Nephrops norvegicus* larvae according to Figueiredo (1971) and Figueiredo and Vilela (1972). The figure represents the time to reach the moult to stage IV. A second-order polynomial function was fitted to the centres of the uncertainty intervals, which are represented by the horizontal and vertical bars, and used to calculate development stage by the model.



(400 m). Moreover, in the uniform scenarios the larvae were randomly placed from the beginning within the respective depth strata, because a migration from the bottom after hatching would have a negligible effect on horizontal transport.

At 12 to 18 °C, which approximates minimum and maximum temperatures to which larvae may be exposed to during winter in southern Portugal, time to reach the stage of megalopa (stage IV, following the three zoeal stages) varies between 10 and 35 days, respectively (Fig. 3). Relative age of each larva was calculated at each time step of the model, based on the number of minutes a larva is exposed to a specific temperature along its track. The number of degrees × minutes necessary to develop through each stage was estimated from a second-order polynomial fit to the data of temperature-dependent development by Figueiredo (1971) and Figueiredo and Vilela (1972). Relative age was set to 0 at release and 1 at moult to stage IV. Once larvae reached age 1, they were assumed to be competent for settlement and were sent to the bottom and their position was recorded and classified in one of sixteen boxes shown in Fig. 4. The boxes are meridionally or zonally organised and also defined in relation to the 800 m isobath (that roughly delimits the slope zone and the lower limit of distribution of adult *N. norvegicus*). Decapod competent larvae may delay moult to the juvenile stage if an appropriate settling substrate is not available (Rothlisberg 2002). To simulate a possible delay in assuming a benthic life, larvae were allowed to drift along the bottom layer for a period of time arbitrarily set to 1/4 of the time they took to reach age 1. Accordingly, position of the larvae was also recorded at age 1.25.

Five simulation trials were run corresponding to the five behavioural scenarios. The runs started on 1 December 2005 and lasted for 4 months. During the first 2 months of each simulation (December and January), larvae were released every day from a region where the highest densities of spawning lobsters have been historically recorded (e.g., ICES 2006), centred at longitude 7.5° W and latitude 36.8° N (see oval inner dark grey region in Fig. 1*b*, centred at those coordinates). The number of particles released per day followed a Gaussian distribution centred in the middle of the release period (1 January) and with standard deviation of 15 days (Fig. 5). The smallest release was of 227 larvae, corresponding to larvae uniformly separated by a distance of 1.5 km in the region of release. The highest release was 10 times this number during the middle of the release period (Fig. 5). A total number of 83 536 larvae were released per scenario, all from the region defined above.

Results

Ocean and atmosphere model results and comparison with observations

A more systematic validation of the numerical configuration described above, involving comparison with observations and with other studies, is provided in Peliz et al. (2007*a*) and Teles-Machado et al. (2007) (another study (Á. Peliz, P. Marchesiello, A.M.P. Santos, J. Dubert, A. Teles-Machado, M. Marta-Almeida, and B. Le Cann, unpublished data) will present detailed analyses of observations and model currents along the slope zone that include our study period). According to these studies, the current system along

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Fig. 4. Division of the studied area (small domain, SD) into 16 boxes where the number of larvae reaching relative ages of 1 and 1.25 was counted. The larvae release location is also shown as the shaded spot centred at 36.8 N, 7.5 W. The 800 m isobath separates the coastal and oceanic boxes.

the Gulf of Cadiz slope is largely determined by the dynamics inherent to the coupling between the Mediterranean inflow and outflow, and to a less degree, to the atmospheric forcing. The authors describe a very low correlation between winds and currents at daily to synoptic scales and conclude that the flow variability depends on other factors, hardly controllable in a model simulation, like the internal variability associated with the chaotic nature of the turbulent flow. In this context, a clear match between point observations and corresponding model simulations results is not expectable. Nevertheless, we provide some comparisons of atmosphere and ocean model results with data recorded by an ocean and meteorology surface buoy located at 36.477 N, 6.963 W (denoted by Cadiz buoy, see location at Fig. 1*b*, over the 450 m isobath). This comparison corresponds to the realistic simulation period December 2005 to March 2006.

Observed and simulated wind components are illustrated (*u* and *v* are positive in the east and north directions, respectively) (Fig. 6). Despite the larger variability in the high frequency band of the observations, the model winds represent the major synoptic variability in the observed period, since the match between both wind components is clear. Unfortunately, the available Cadiz buoy record of ocean data only partially overlaps that of the simulation (December 2005 and first week of January 2006). The observed and modelled zonal (*u*, Fig. 7*a*) and meridional (*v*, Fig. 7*b*) surface (3 m) current components are presented. Buoy temperature at 3 m (thick line), satellite-derived sea surface temperature (from Satellite Application Facility (Ocean) (SAFO) database, SAFO (2007)) interpolated at the Cadiz buoy location, and model surface temperature for the same location are illustrated as well (Fig. 7*c*). In what concerns the surface temperature, the model follows the observed values, and the differences are within a range of less than 0.5 °C. The comparison of the model and observed currents shows a fair agreement despite the fact that the observed point is over the slope and relatively far from the shelf. As referred above, the wind-driven component of the currents is reduced, and part of the circulation is due to the internal current variability, which is not predictable, and some of the flow events are not registered or are not in phase in the model and in the observations. For example, the reversals in the north current component by mid-December and beginning of January in the observations time series (Fig. 7*b*) is not in phase with any wind event that could justify such current reversal (Fig. 6). Thus, this reversal in the observed currents is most possibly associated with the meandering and eddy events of the slope system and not with changing wind forcing. This appears to be the reason why in the model simulation these events were not observed in those particular dates. However, they are common in other periods (for a deeper discussion of this subject, see Peliz et al. (2007*a*)).

Main circulation features in the study region

The Gulf of Cadiz constitutes the zone of exchange with the Mediterranean Sea through the Strait of Gibraltar, and

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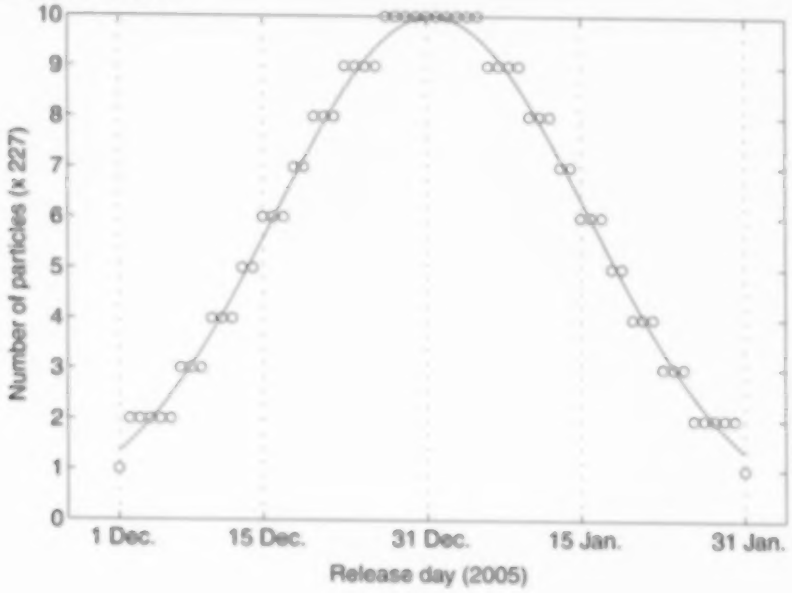
Fig. 5. Number of larvae released per day in each scenario. Larval release followed a Gaussian pattern centred between beginning of December and the end of January, with standard deviation of 1/4 of this period. Total number of larvae released daily varied between 227 and 10 × 227, yielding a total number of 83 536.



the Mediterranean outflow strongly influences the local circulation, particularly the subsurface flow owing to the presence of the MU. The MU is a bottom-trapped plume of about 100 m thickness, generated by the mixing of Mediterranean outflow waters with the central Atlantic water. The MU is strongly forced by the topography from its generation point until the western part of the Gulf, where the plume reaches a buoyancy equilibrium and starts spreading laterally. Still in the eastern part, the plume splits in two main veins, which after stabilization typically remain centred at depths of 600 to 1200 m, with core velocities in the order of 0.3–0.4 m·s^{-1}, transporting warm and salty water from the Mediterranean Basin into the Atlantic Ocean (Baringer and Price 1997; Serra et al. 2005; Peliz et al. 2007*a*).

The model-averaged (December 2005 and January 2006) vertical structure of the alongshore velocity off the southern Portuguese coast, at 7.5 W, is shown (Fig. 8, see the location of the vertical section in Fig. 1*a*; negative (broken lines) and positive (solid lines) values indicate westward or eastward flow, respectively). The MU is present near the bottom and extends to the upper slope, surfacing between 36.6 N and 36.8 N, with westward velocities near 0.1 m·s^{-1} close to the surface.

The complex processes of adjustment of the Mediterranean inflow and outflow are not only important for the bottom currents but also determine the surface and upper slope circulation (Peliz et al. 2007*a*). The MU westward flow is bordered by two eastward (positive) currents (Fig. 8). Offshore, a broad and deep current is driven in association with the mass balance necessary for the entrainment within the MU (a recirculation feature). This flow is observable in the horizontal mean current structure (Fig. 9) as a slope-oriented current with the axis along 36.2 N noticeably east of 8 W and recirculating to the north near the slope where it merges with the MU. Inshore and up-slope of the MU (Fig. 8), a second jet-like feature shallower than 200 m with typical transport of 0.4 Sv (1 Sv = 10^6 m^3·s^{-1}) and velocities of around 0.15 m·s^{-1} is observable. This second equatorward flow is driven by the the inflow of surface Atlantic waters into the Mediterranean Basin, and it is part of a continuous slope equatorward current (denoted by Gulf of Cadiz Current (GCC) in Peliz et al. (2007*a*). The GCC extends from Cape St. Vincent to the Strait of Gibraltar and intensifies east of Faro (see the inshore-most equatorward flow in Fig. 9). Observations of this upper slope current are reported in Garcia-Lafuente et al. (2006) and Garcia Lafuente and Ruiz (2007).

In summary, the circulation in the Gulf of Cadiz is constituted of a bottom-intensified westward flow (the MU) with little but substantial transport at the surface. Offshore, a broad and deep flow is generated in response to the MU, promoting a eastward transport at all depths. Inshore and up-slope of the MU, a second narrow but intense current (the GCC) dominates the upper slope and outer shelf circulation, inducing also a eastward (equatorward) transport. Peliz et al. (2007*a*) (also Á. Peliz, P. Marchesiello, A.M.P. Santos, J. Dubert, A. Teles-Machado, M. Marta-Almeida, and B. Le Cann, unpublished data) thoroughly discuss this circulation scenario and compare it with observations and with other theoretical models. The authors argue that in mean terms, theses circulation features are very persistent and little influenced by the local wind forcing. Thus, they are determinant for transport and dispersal processes with time scales larger that the atmospheric synoptic forcing (a few days to 1 week)

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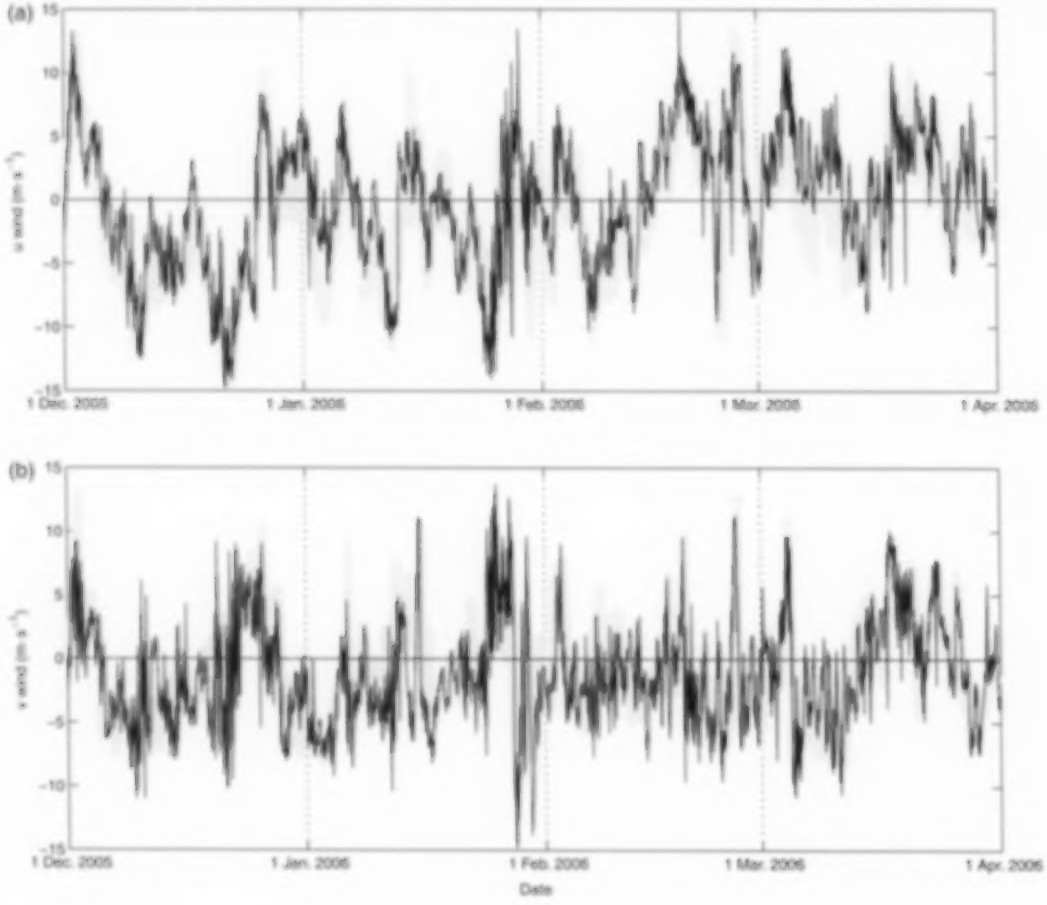
Fig. 6. (*a*) Zonal (*u*) and (*b*) meridional (*v*) components of the wind at the Cadiz "Puertos del Estado" buoy (thin black line) and atmospheric model WRF (i.e., weather research and forecast, thick grey line) for the simulations period. Both represent raw data with samplig rates of 1 h (Cadiz Buoy) and 4 h (WRF).



and larger than the typical ocean mesoscale coastal and slope eddy events (several days to 1 or 2 weeks), which match those we are interested in the present study.

General patterns of larval dispersion

The main strategy for the analysis of the IBM results was to register the number of individual particles arriving at given sites after reaching ages 1 and 1.25. This result is interpreted as a proxy for the probability of larvae originating at the release location to settle in that particular area. Since we wished to analyse the actual distribution of settlement over the whole domain, we did not standardize number of larvae per unit area. The number of larvae reaching ages 1 and 1.25 per day (during the simulation period), and arriving at boxes 5 to 16, is illustrated (Fig. 10). Boxes 1 to 4 are not shown because the corresponding values were always zero or very low. The plots are organised into matrices, where the rows refer to boxes and the columns correspond to the five different migration scenarios.

The integrated information for the whole simulation period is shown. For each box, a bar diagram is displayed representing the percentage of larvae that reached age 1 (Fig. 11*a*) and age 1.25 (Fig. 11*b*) in that particular box. Each bar in the diagrams corresponds to the results of a given experiment as has been used for Fig. 10. For example, the sum of the first bar in each diagram corresponds to the 100% of larvae counted inside the 16 boxes. Only a small fraction (less than 5%) of the whole number of larvae abandoned the SD (boxes 1–16) through Gibraltar Strait or the western and southern boundaries.

The majority of larvae reached age 1 or 1.25 along the southern coast inshore of the 800 m isobath (boxes 12, 14, and 16) and in particular in box 14 where larvae were hatched (Fig. 4). This can be seen by summing the percentages given in Fig. 10*b* for boxes 12, 14, and 16 separately for larvae aged 1 and 1.25. These totals ranged from 58.3% to 94.6% in the case of U5, U50, U200, and DVM200 scenarios and were 44.7% and 39.9% in the case of the DVM400 scenario. The only substantial concentrations of larvae offshore of the 800 m isobath were found in the southwest region, mainly in box 9, which corresponds to a larger oceanic area. Another important spatial pattern is that

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Fig. 7. (*a*) Zonal (u) and (*b*) meridional (v) components of surface velocity measured by the Cadiz buoy current meter (thin line) and the surface velocity resulting from the ocean numerical model (thick grey line). (*c*) Comparison of sea surface temperature of model (continuous grey line), buoy (continuous thick line), and Satelite Application Facility (Ocean) (SAFO) database (discontinuous thin line) for the period 1 December 2005 to 6 January 2006. All represent raw data with sampling rates of 1 h (Cadiz buoy), 0.3 h (model data), and 8 h (SAFO temperatures).

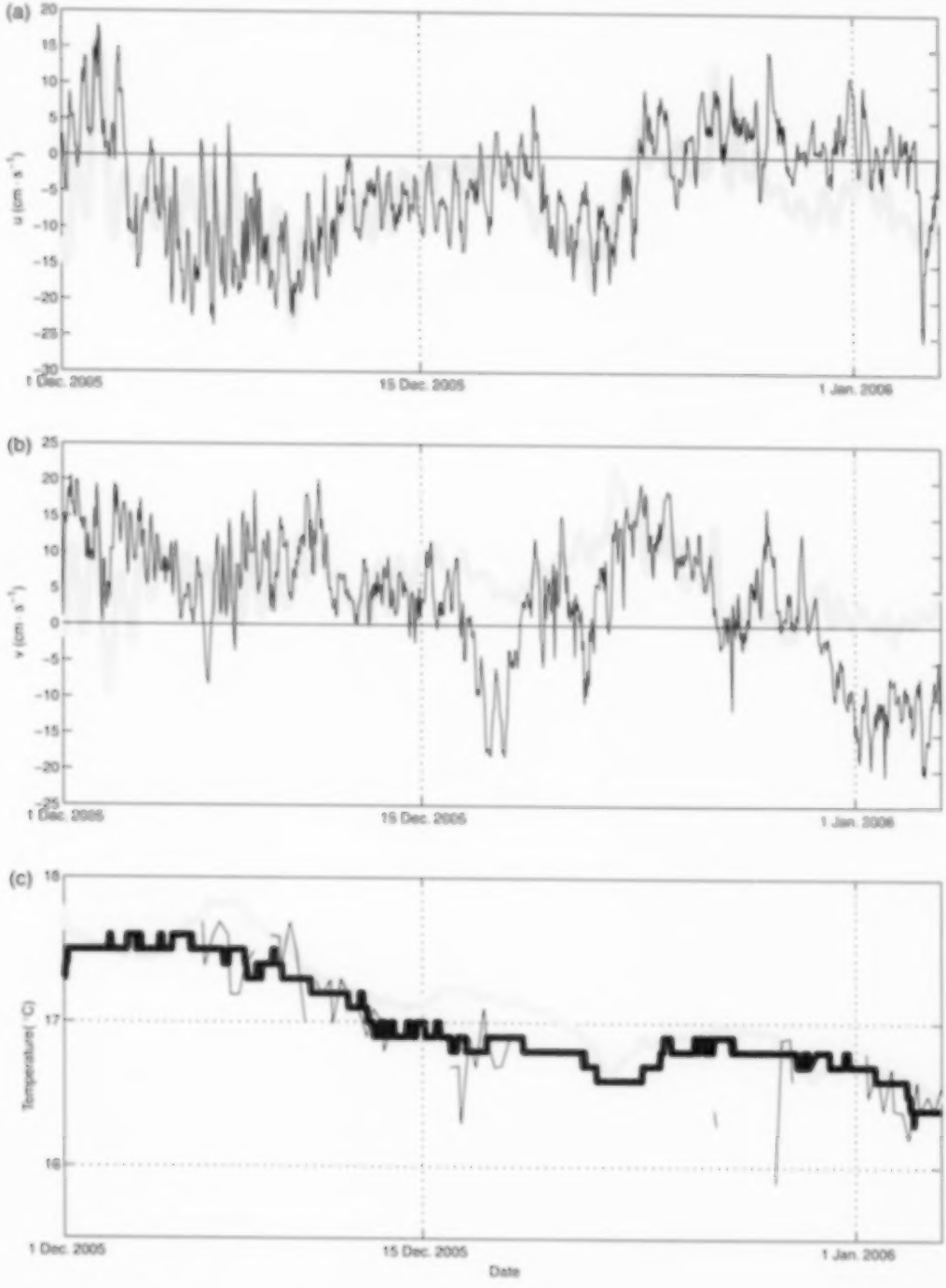

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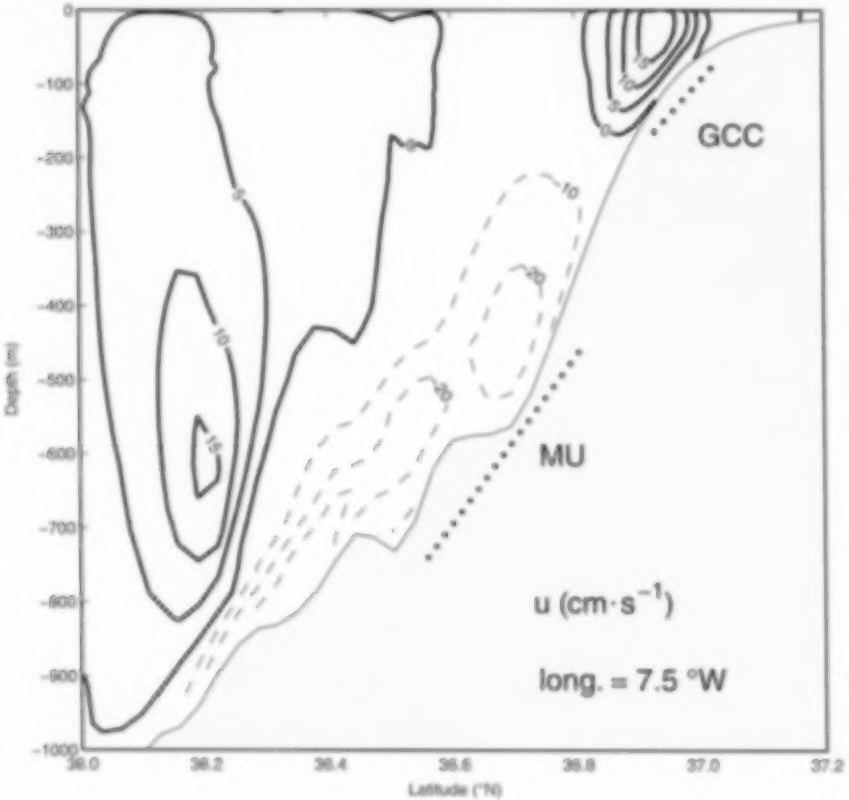
Fig. 8. Mean (December 2005 and January 2006) alongshore velocity at the southern Portuguese coast, at 7.5 W (negative values for broken lines, positive values for solid lines indicate westward or eastward flow, respectively) resulting from the model output. See location of the slice at Fig. 1*b*. MU, Mediterranean undercurrent; GCC, Gulf of Cadiz Current.



coastal regions off the west coast, north of Cape St. Vincent, received very low numbers of larvae. The sum of percentages in boxes 6 and 8 ranged from 0.0% to 2.2% in the case of uniform and DVM200 scenarios in ages 1 and 1.25 larvae and were 7.5% in the case of the DVM400 scenario for both ages (Fig. 10*a*). North of 38°N (boxes 1 to 4), the number of larvae in the inshore and offshore boxes was very low, typically less than 0.1% of total larvae.

It is worth noting that in spite of box 10 being about two orders of magnitude smaller than box 9 and also smaller than the box adjacent to the east (box 12), the number of larvae that settled there was relatively high, ranging from 0.5% to 6.5% of the larvae released depending on the behavioural scenario and age (Fig. 10*a*). Conversely, the percentage in the area adjacent to the north of box 10 (box 8) was rather low. This suggests box 10 as a passage to western, and to a less extent, northern regions, as verified by analysing the trajectories of the particles (not shown). In boxes 11, 13, and 15, south of Gulf of Cadiz, small numbers of larvae with ages 1 or 1.25 were observed, indicating weak southward larval transport.

Differences between migration scenarios

The most striking difference between uniform and DVM scenarios was observed around the release area (boxes 14, 16). While passive larvae without DVM (i.e., uniform scenarios, U5, U50, and U200) were generally retained or advected eastward toward the Gibraltar Strait, larvae subjected to DVM (DVM200 and DVM400) were dispersed westward and beyond the slope (Fig. 11). In the eastern onshore regions (boxes 14 and 16), an increase of the larval depth range (U5 to DVM400) is associated with a decrease in larvae abundance. In general, the trend for the larvae to stay in deeper layers implied a decrease of larvae in boxes 14 and 16. The opposite behaviour was observed for the western regions, namely boxes 9 and 10 (i.e., the number of larvae increased; Fig. 11). This behaviour is a consequence of the circulation described above, with deep currents of the MU flowing westward (Fig. 8), and the surface GCC flowing in the direction of Gibraltar (Fig. 9).

A bimodal pattern regarding the number of larvae reaching age 1 (but also valid for age 1.25) was observed, mainly in the eastern coastal boxes 14, 16, and to a less extent in box 12), for the uniform scenarios (Fig. 10*b*). The first peak around 10 January originated from the release of particles in relatively warm temperature waters (16.5–17 °C) during the 2 preceding weeks (see Fig. 7*c*). After that period, sea water temperature decreased about 1.3 °C (from 1 January to 1 February). Thus, the time to reach age 1 increased from about 2 weeks (Fig. 3) to about 3 weeks. The second peak

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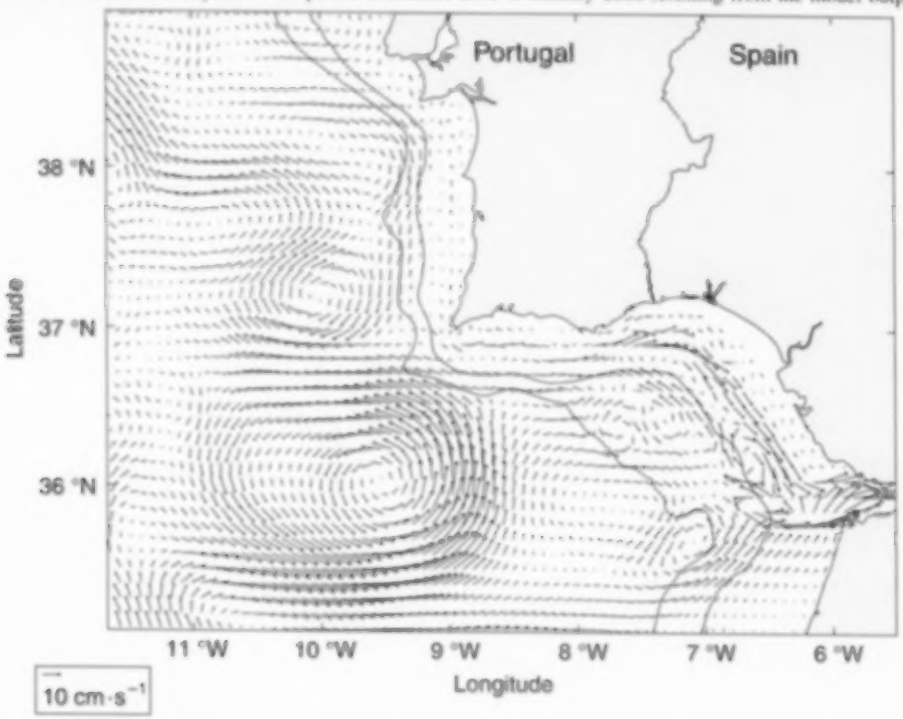
Fig. 9. Mean surface velocities (20 m depth) for the period December 2005 to January 2006 resulting from the model output.



may be explained as the time of response to the maxima of hatched larvae (i.e., corresponds to larvae released around 1 January). For the U200 behavioural scenario, the bimodal peaks were not observed because a relatively large number of larvae were isolated from the surface. Note that below the surface mixed layer, the temperature is nearly constant in time. Thus, in scenarios DVM200 and DVM400, the same behaviour as U200 was observed.

Another important difference between the uniform and DVM scenarios concerns the exchange of larvae to regions north of 37 N. While DVM400 larvae were found in relatively high numbers in boxes 5, 6, 7, and 8, larvae behaving according to uniform scenarios were almost always absent in these areas (with the exception of U200). This can be easily observed in the spatial distribution of larvae by 1 February, 1 month after the release maxima (Figs. 12*a* and 12*c*).

Comparison of ages 1 and 1.25

The time series of the number of larvae becoming adult show a displacement of about 1 week between the time to reach ages 1 and 1.25 (Fig. 10). This is not a surprising result because 0.25 units of age represent about 1 week for a temperature of 14 °C (Fig. 3). At age 1.25 (Fig. 11*b*), since the larvae from all behavioural scenarios are forced to remain in the bottom layer from age 1, they are expected to predominantly move in the westward direction. For instance, in boxes 9 and 10 the number of larvae is strongly increased for uniform scenarios at age 1.25 (Fig. 11). In box 9 the increase is from less than 1% to about 14% in scenarios U5 and U50. As result, there was a decrease in larvae that reached age 1.25 in the onshore regions of the south coast in all scenarios, relative to age 1 larvae. This again can be seen by summing the percentage of larvae reaching ages 1 and 1.25 in boxes 12, 14, and 16 (Fig. 10*b*). While in the case of age 1.25 larvae the values ranged from 39.9% to 79.2%, they ranged from 44.7% to 94.6% in the case of age 1 larvae. This behaviour is due to the advection of larvae by the MU, and larvae are exported (and lost) to the open ocean (box 9). The spatial distribution of larvae with ages 1 and 1.25 by 1 February (Fig. 12) is representative of the general patterns of larval dispersal. For uniform scenarios in Figs. 12*a* and 12*b*, the comparison of age 1 with age 1.25 indicates a clear westward transport due to MU. For DVM scenarios in Figs. 12*c* and 12*d*, such a clear difference between ages is not observed because the larvae are influenced by the MU during the entire life history.

Discussion

The spatial patterns of distribution of competent larvae predicted by the simulations can be explained by the circulation features off the south coast of Portugal. While the deeper layers are dominated by the westward-flowing MU (Ambar and Howe 1979), near the surface the GCC (Peliz et al. 2007*a*) flows eastward over the upper slope towards the Strait of Gibraltar. Moreover, circulation over the shelf areas shoreward of the main core of the GCC is directly controlled by the meridional component of the wind, which shows periods of 10–15 days dominated by negative or positive values, resulting in currents flowing alternately westward and eastward. The interaction of the MU and

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Fig. 10. Number of larvae reaching relative ages of 1 and 1.25 in boxes 5 to 10 (*a*) and 11 to 16 (*b*). Each row of plots represents one box, and the columns represent the behavioural scenarios. The thin line in each plot is the time series of larvae at age 1, starting at the beginning of the simulation period (1 December 2005). The light-font number represents the percentage of the total larvae released that reached age 1 in the corresponding box. Bold lines and numbers represent the same for larvae at age 1.25.

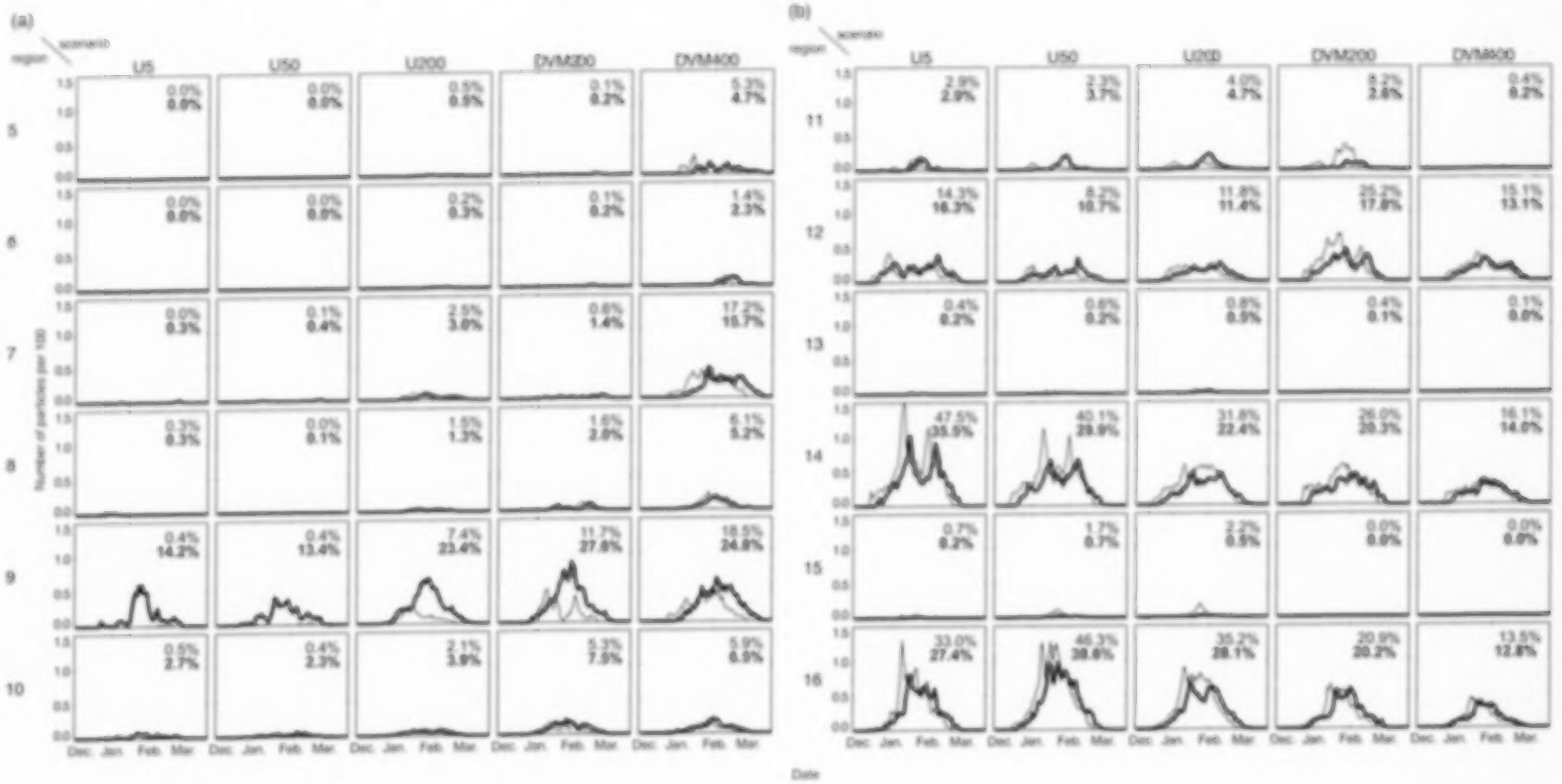

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Fig. 11. Percentage of total larvae reaching relative ages of 1 (*a*) and 1.25 (*b*) in each of the 16 boxes. The groups of five bars represent the five behavioural scenarios (from left to right): U5, passive larvae (no diel vertical migration (DVM)) released uniformly within a 5 m thick surface layer; U50, passive larvae released uniformly between the surface and a depth of 50 m; U200, passive larvae released uniformly between the surface and a depth of 200 m; DVM200, larvae undergoing diel vertical migration between the surface and a depth of 200 m; DVM400, larvae undergoing diel vertical migration between the surface and a depth of 400 m.

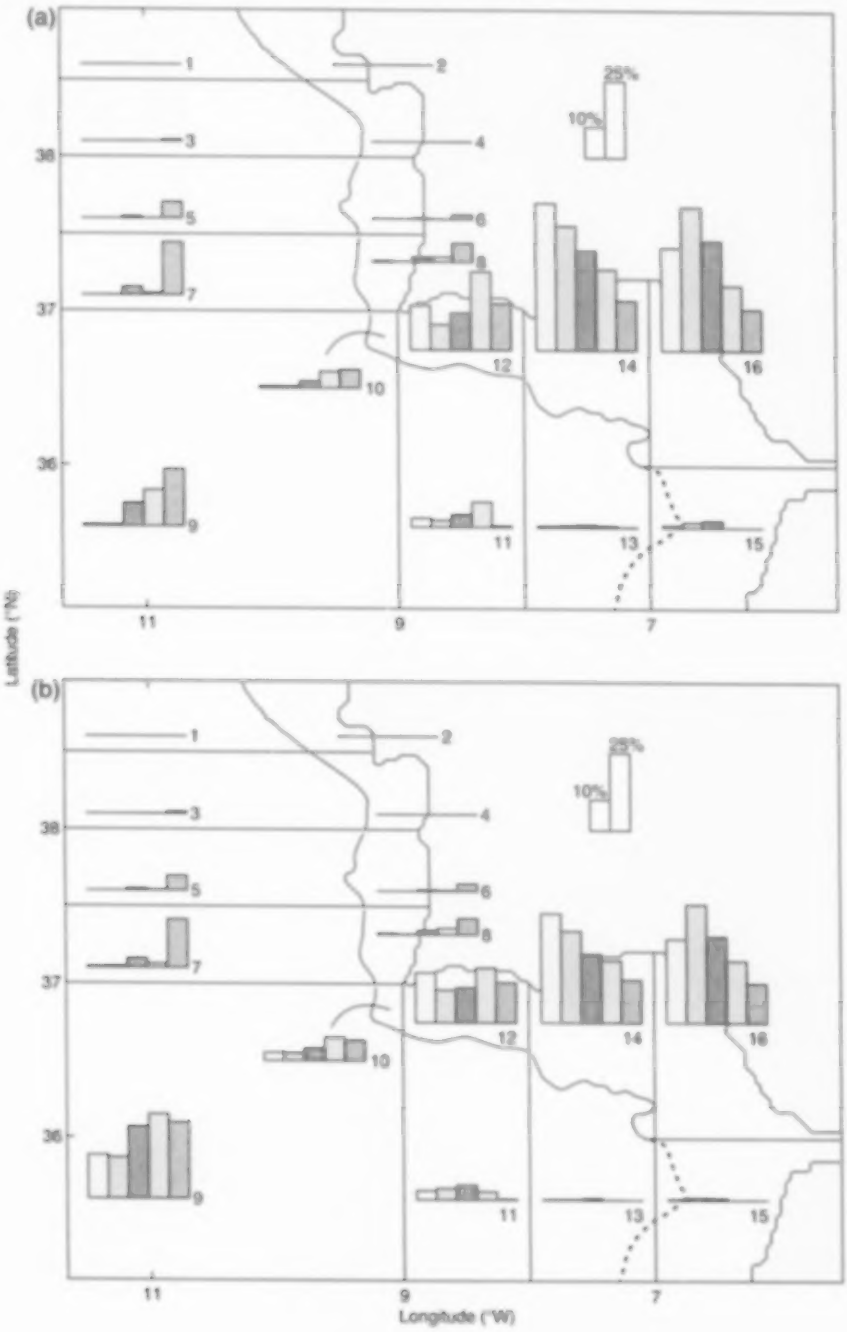

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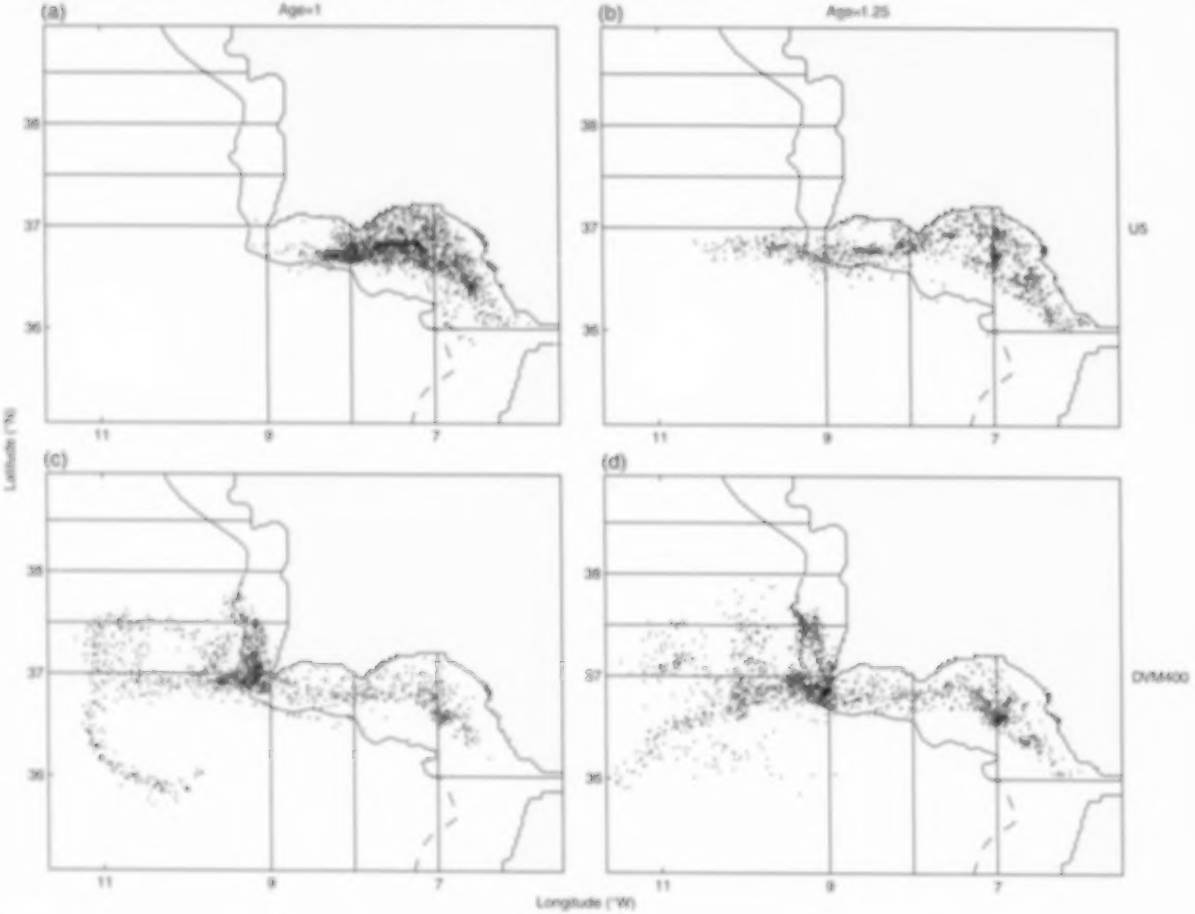
Fig. 12. Spatial larval distribution on 1 February for the scenarios U5 (passive larvae (no diel vertical migration (DVM)) released uniformly within a 5 m thick surface layer; top row) and DVM400 (larvae undergoing diel vertical migration between the surface and a depth of 400 m; bottom row). Panels *a* and *c* refer to age 1; panels *b* and *d* refer to age 1.25.



GCC flows with the coastal topography, the bathymetry, and with each other results in pairs of cyclonic and anticyclonic eddies. These eddies are transient structures but tend to be recurrent at the same locations, leaving a strong signature on the mean circulation (Peliz et al. 2007*a*). The most important pair is located to the west and south-southwest of Cape St. Vincent, but smaller structures are also present in the Gulf of Cadiz. Accordingly, the retention of the larvae in the onshore regions of the south coast, where the largest densities of adults are found, can be interpreted as resulting from the interaction of the GCC with the coastal wind-driven circulation, and the probability of retention in this general area increases, as the behavioural scenarios predict a shallower distribution of the larvae. Larvae transported to the central Gulf of Cadiz (boxes 11, 13, and 15) and to the offshore areas of the west coast (boxes 5, 7, and 9) will be trapped by the gyres or channelled offshore and lost from the system. The possibility remains that some of the larvae can be transported to the coastal areas of the west coast and settle there. The simulations show that very few larvae remaining in the upper 50 m will be transported there, but that a deeper distribution increases the probability of northward transport around Cape St. Vincent.

The values presented above for the percentage of larvae advected to the different regions allow an assessment of the general patterns of *N. norvegicus* larval dispersal in the area. These values do not include the effect of larval mortality. Mortality values currently reported in the literature for invertebrate larvae range from 0.02 to 0.82 per day (Morgan 1995). These values were generally obtained by following a cohort of larvae through space and recording self-thinning or by integrating abundances of successive stages over a given area. In both cases, these values apply to mortality during the planktonic life and, as such, do not include mortality from failing to find an appropriate settlement substrate or habitat. Additionally, both methods do not consider the distribution of mortality factors over the large geographical span of many populations, and the integration method does not usually account for losses due to advection from the area (Helbig and Pepin 1998). Nevertheless, the current consensus is that the majority of larvae, in many cases more than 90%, will die before starting the search for an appropri-

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ate substrate to settle. Thus, the combined effects of mortality due to disease, starvation, predation, and wastage from appropriate habitats has a decisive effect on the dynamics of marine populations (Gaines and Lafferty 1995; Caley et al. 1996; Morgan 2001). A tentative estimate of the proportion of *N. norvegicus* larvae hatched from the area of high density of adults that self-recruit into that same area can be obtained by (*i*) calculating the number of larvae that settle in that area and (*ii*) using a survival estimate of 0.15 (from hatching to stage III for larvae in the Irish Sea) (figure 9 and table 5 in Dickey-Collas et al. 2000), which is a conservative value given that it does not account for mortality during stage III. Scenarios U50 and DVM400 were those that resulted in the largest and smallest self-recruitment, with values of 3.2% and 1.0%, respectively. Applying a survival estimate of 0.15 to the case of age 1 larvae under these extreme scenarios indicates that only 0.5%, if they remain in surface waters, to 0.2%, if they migrate between the surface and 400 m, of the larvae hatched would successfully self-recruit.

Generalizations of this level of recruitment to the whole region covered by the Algarve stock must be made with caution because this estimate does not consider possible subsidies form neighbouring populations, which are not necessarily identical in their capacity to act as sources or sinks of larvae (Fogarty 1988; Armworth 2002). Nevertheless, these estimates highlight the potential for dispersal of *N. norvegicus* larvae away from the hatching sites in the environmental setting of southern Portugal and show that a considerable proportion may be lost to deeper areas of the open ocean where settlement success is very unlikely. The results of the simulations also show that an important proportion of larvae hatched from the Algarve stock may recruit into the small stock off Cape St. Vincent and that a smaller fraction may reach the stock on the west coast off Sines. Whether these contributions of the Algarve stock in terms of self-recruitment and recruitment subsidies to other local populations have any demographic meaning, in the sense that they are enough to compensate for local mortality (Cowen et al. 2006), cannot be answered by the present study. However, it appears that transport of *N. norvegicus* larvae over distances of 100–300 km along the south and southwest Atlantic coasts of the Iberian Peninsula is possible and that these populations may be genetically connected.

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Escapement goal analysis and stock reconstruction of sockeye salmon populations (*Oncorhynchus nerka*) using life-history models

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Abstract: We compare life-history models with the Beverton–Holt approach of escapement goal analysis. We model the life history of a sockeye salmon (*Onchorhynchus nerka*) population from a spawning stage, through juvenile and adult stages, and ending with adults that return to spawn. We fit models to data by statistically comparing predicted and observed numbers of four dominant adult ages. Posterior estimates of parameters from Markov chain Monte Carlo simulations are then used to assess optimal harvest policies. We search for policies that produce the highest average yield. We find that it is possible to detect density dependence with a life-history model where analysis of Beverton–Holt stock–recruitment relationship fails to do so. We find that Beverton–Holt relationships produce policies and long-term yield estimates that are inconsistent with empirical trends. Conversely, we find that optimal spawning stock sizes and maximum sustained yield estimates using the life-history model estimate are consistent with the historical behavior of fisheries examined. Adding smolt data to the analysis does not substantially change predicted optimal spawning stock size, but decreases the variance in estimated posterior parameter distributions and policy variable distributions.

Résumé : Nous comparons des modèles démographiques à la méthode Beverton–Holt pour l'analyse des objectifs de l'échappement. Nous avons modélisé le cycle biologique d'une population de saumons rouges (*Oncorhynchus nerka*) à partir du stade de la fraie, en passant par les stades juvéniles et adultes, pour finir avec les adultes qui reviennent frayer. Nous avons ajusté les modèles aux données en comparant statistiquement les nombres prédits et observés de quatre classes d'âge dominantes d'adultes. Nous avons ensuite utilisé les estimations a posteriori des paramètres provenant de simulations de type Monte-Carlo avec chaînes de Markov pour évaluer les politiques optimales de récolte. Nous recherchons les politiques qui produisent le rendement moyen maximal. Nous observons qu'il est possible de détecter la densité-dépendance avec un modèle démographique, ce qui n'est pas possible avec une analyse des relations stock–recrutement de Beverton–Holt. Les relations Beverton–Holt mènent à des politiques et des estimations du rendement à long terme qui sont incompatibles avec les tendances empiriques. À l'inverse, les estimations de la taille optimale du stock reproducteur et du rendement maximal durable produites par le modèle démographique sont en accord avec le comportement passé des pêches commerciales examinées. L'addition des données sur les saumoneaux à l'analyse ne change pas de façon importante la prédiction de la taille optimale du stock reproducteur, mais elle réduit la variance de la distribution estimée des paramètres a posteriori et des variables reliées à la gestion.

[Traduit par la Rédaction]

Introduction

Since the 1950s and Ricker's classic paper (Ricker 1954), the most common way of evaluating the performance of salmon stocks has been to aggregate all fish from single broods that return to spawn into units of recruitment and examine those recruitments across the range of spawning numbers that produced them. Optimal harvest strategies are then found by calculating exploitation rates or fixed spawning biomasses that maximize yield. While it makes for a parsimonious stock–recruitment model, it does not allow us to attribute observed variation to known stages of life history. Partitioning life-history stages into recruitment from one stage to another can produce a variety of functional forms (Paulik 1973). In this paper, we evaluate whether additional insight can be gained from dissecting the process into distinct life-history stages and predicting recruitment arising from fish having survived through stages of life history that have different survival–density relationships. We develop a suite of life-history models (LHMs) to examine the feasibility of interpreting historical trends, inferring biological process, and developing alternative policy options.

Salmon escapement goal analysis almost always begins with a mathematical relationship between spawning stock and subsequent total adult returns (Hilborn and Walters 1992). In the absence of spawner–recruit data, habitat-based methods have been used (Geiger and Koenings 1991). Analyses of trends in productivity primarily look at the trends in brood year recruits per spawner (Peterman et al. 2000, 2003). Adkison et al. (1996) showed that there were persistent changes in productivity throughout the 1970s, indicating that perhaps spawner–recruit relationships might account for the variability. The drawback of setting escapement goals

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with simple spawner–recruit relationships is that variability in density-dependent survival will be averaged across stages of life history. When the data are compared across the entire life history, survival–density relationships that would be visible within a particular stage of life history are lost. In some cases, the averaging across many stages of life history poses no problem because nearly all fish follow the same life history, but this is not always the case. Studies have shown that it is not optimal to adjust spawning stock sizes to temporal variation in productivity when it is unpredictable (Walters and Collie 1988) and that constant harvest rate policies can compensate for the effect of interannual variation in productivity (Mace 1994; Walters and Parma 1996). We propose that a more detailed analysis of life histories can aid in the development of more robust policies in the face of uncertainty and changing environmental conditions.

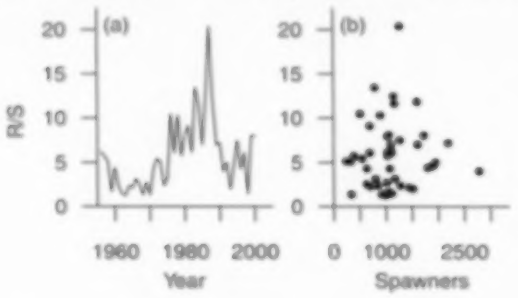
Classic stock–recruitment theory assumes aggregated survival rates across life-history stages; however, not all adults have spent equal amounts of time at those life stages. Furthermore, if much of the variability in returns is due to survival in the year of smolt migration, aggregating data into brood years ignores that relationship. The consequences of aggregating information across age classes is most pronounced in stocks with complex age structures like Bristol Bay sockeye (*Oncorhynchus nerka*). Regime shifts (Beamish and Bouillon 1993; Hare and Francis 1995; Adkison et al. 1996) further confound identification of productivity relationships with decadal patterns visible in both catch and production.

Age-class structure of individual sockeye stocks varies regionally. Typically, 95% of Fraser River sockeye spend 1 year in lakes and 2 years in the ocean before returning to spawn (Cass and Wood 1994), predominantly a single age class for all Fraser stocks. Conversely, roughly 95% of Bristol Bay sockeye are distributed among four age classes rather than just one, spending either 1 or 2 years in lakes before smolting and 2 or 3 years in the ocean before returning to spawn. Among Bristol Bay stocks, the distribution of these age classes also varies considerably.

Ocean survival can be inferred from spawner abundances from the same brood returning in different years. Much of the variation in salmon returns appears to be due to changes in ocean survival, which also proves to be the dominant covariate among Fraser and Bristol Bay sockeye stocks (Peterman et al. 1998). However, since ocean entry timing affects the body size of smolts, a critical determinant of survival during the most critical period of life (Koenings et al. 1993), brood year recruits per spawner is not a good measure of marine survival. For stocks of pink (*Oncorhynchus buscha*) and chum (*Oncorhynchus keta*) salmon and many stocks of coho (*Oncorhynchus kisutch*) and Chinook (*Oncorhynchus tshawytscha*), all individuals from the same spawning brood migrate to sea at the same time, and brood year recruits per spawner will reflect those changes in marine survival. This is not the case when there is variable smolt timing and variable spawner migration timing, such as is the case for Bristol Bay sockeye.

If there are systematic changes in ocean survival, it is difficult to distinguish whether variability in ocean survival or density-dependent effects in fresh water are responsible for the changes in recruits per spawner; density dependence

Fig. 1. Recruits per spawner (*R/S*) plots of the Egegik system. Panel (*a*) shows time series of *R/S*, and panel (*b*) shows *R/S* vs. *S*.



being the focal point of maximum sustained yield calculations. An example is the increase in recruits per spawner in the Egegik stock of Bristol Bay sockeye salmon. Recruits per spawner increased dramatically in the late 1970s (see Fig. 1*a*), a change generally ascribed to the ocean change known as the Pacific Decadal Oscillation (PDO) (Mantua et al. 1997). Plots of recruits per spawner vs. spawners show little evidence of density-dependent recruitment compensation (see in Fig. 1*b* that there is no apparent negative slope). However, biologists working in the system have recognized that as ocean survival increased and returns were larger, the average spawning stock also was larger, implying that higher ocean survival led to larger spawning stocks, rather than larger spawning stocks leading to higher recruits per spawner.

To account for changes in ocean survival, the approach has been to analyze the data in blocks of time, corresponding to different ocean regimes, and assume that density-dependent and -independent rates of survival were constant within blocks of time, but not necessarily among blocks of time. For instance, the most recent escapement goals for Egegik used the data from brood years 1978–1993. There are obvious problems in deciding what block of years to use. It appears that the pulse in high recruits per spawners ended about 1990 (see Fig. 1*a*). Treating high production years as anomalous and excluding those years from the analysis can underestimate productive capacity, particularly in systems where peak years account for a large portion of long-term yield.

Moussalli and Hilborn (1986) showed how a model in which each stage was represented by a Beverton–Holt curve could be used to evaluate the impact of changes in carrying capacity or productivity at each life stage on the sustainable yield and optimal escapement. In principle, with data on abundance at each life stage, such models could be fit to data for fish stocks and density dependence at each life stage, and changes in productivity could be estimated. It is rare to have data on more than escapement and adult return; however, for some stocks, such as the Egegik, Kvichak, and Ugashik systems, there are estimates of smolt counts, relative smolt abundances, or at least smolt age composition. A multistage model can be used to fit whatever data can be attributed to one of the stages. In this paper, we make an initial step in this direction, showing how a model that considers freshwater life history and density dependence, variable freshwater residence time, and explicit ocean survival

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Table 1. Key differences between stocks, including average and maximum run sizes (in millions) and availability of smolt data (Juvenile).

Stock	Average	Maximum	Juvenile	Population	Production
Ugashik	2.2	7.8	Yes	↑	↑
Egegik	5.2	25	Yes	↕	↑
Kvichak*	9.6	47.7	Yes	↓	↓
Wood	2.6	6.2	No	NC	NC

Note: Arrows indicate the relative change in the population size and production of the stock in recent years; double-ended arrow indicates both increases and declines have been observed in population. NC, no change.

*Cyclic stock.

and maturity rates can be used to evaluate trends in ocean productivity and optimal escapement. Potential advantages to this include estimating trends in ocean survival by year of ocean entry, using data from other stages than adult returns (i.e., smolt abundances), the ability to relate environmental covariates specifically to stages of life history, and the ability to build intercohort interactions into population dynamics. Predominantly, we focus here on optimal escapement.

Further, we can imbed this explicit life-history model in modern statistical approaches for model choice (Burnham and Anderson 2002) to explore models of varying levels of complexity, where parameters such as freshwater survival rates of specific ages of fry, smolt migration rate, ocean survival rates, and spawning migration rate may be considered time-variant or constant across all years. There are many degrees of biological complexity that can be incorporated into such a model, but in this paper we concentrate on the relative benefits of accounting for interannual differences in freshwater and ocean survival rates. Our primary concern is to develop a method for detecting density dependence from spawner recruit data. We contrast case examples where such models are applied.

Materials and methods

Data

Bristol Bay is located in the south–west of Alaska (58 N, 160 W). Historical catch and escapement data from Bristol Bay commercial fisheries were provided by the Alaska Department of Fish and Game (333 Raspberry Road, Anchorage, AK 99518, USA). Escapement counts were obtained from towers located on the banks of rivers. Seasonal escapement values were obtained by expanding the number of fish counted in a 10 min period to a 60 min total and summing the daily totals to a single total count at the end of the season. Catch numbers were obtained by sampling from the deliveries to processors to get the number of fish per unit sampled weight and then calculating total number from total weight of delivered fish. Age composition was obtained from scale analyses of samples obtained from commercial catches and escapement. We assumed a combined observation error in catch and escapement (i.e., the total returns). Smolt numbers come from calibrating sonar readings to samples obtained from fyke nets. Total smolt numbers were apportioned into age classes using the ratios obtained from the samples. Where smolt counts were used, we assumed an observation error in the estimated number, but no error in the ratio of smolt ages estimated. We further assume that fish counted at escapement towers are fish that originated from that river system and that catch sample analysis allocates fish caught to the system from which they originated. We emphasize that numbers treated as data in this analysis are in fact calculated from statistical models applied to the sample data. This in no way diminishes the value of the information. The statistical methods we employ formally treat these biases as though the data were observed with some error that follows some statistical form, regardless of whether it is human error or error propagated by the stock reconstruction methods. We emphasize that many of the fine details of data collection and data processing contribute to the cumulative statistical noise that we account for in our methods as an aggregate human observation error. We analyze the catch and escapement numbers for four systems: the Ugashik, Egegik, Kvichak, and Wood river systems. Each one brings to bear unique ecological and management conditions for the sake of contrasting the type of modeling framework proposed herein. They represent distinct geography relative to fisheries in Bristol Bay, as well as different historical levels of production, availability of smolt data, and range of variation in run size. (Table 1).

Model

We developed a life-history model of sockeye in which we allow for 1 or 2 years of freshwater lake residence and 2 or 3 years of ocean residence. The age groups are labelled "freshwater years.ocean years" (Koo 1962) (i.e., 1.2, 1.3, 2.2, and 2.3). Since the egg stage is not accounted for in this convention, 1 year must be added to reach the calendar year return (e.g., 1.2s return 4 years after the spawning year). A system of equations (eqs. 1–12) describes transitions between life-history stages. A graphical representation of the life history is provided (Fig. 2) along with a List of symbols.

We assume eggs produced in year y are the product of adult returns and a fecundity rate per adult regardless of sex ratio.

$$(1) \qquad E_y = S_y F$$

We assume a Beverton–Holt (BH) relationship between eggs deposited and fry production. We use the BH functional form because biologically, it implies a survival rate declining to zero at the value of it's fry production capacity parameter (C). It allows us to translate fry capacity to adult capacity by scaling to the survival rate to adulthood.

$$(2) \qquad J_{0,y+1} = \frac{E_y}{\frac{1}{\lambda_0} + \frac{E_y}{C}}$$

The abundance of fry that survive to the ath year in the lake is

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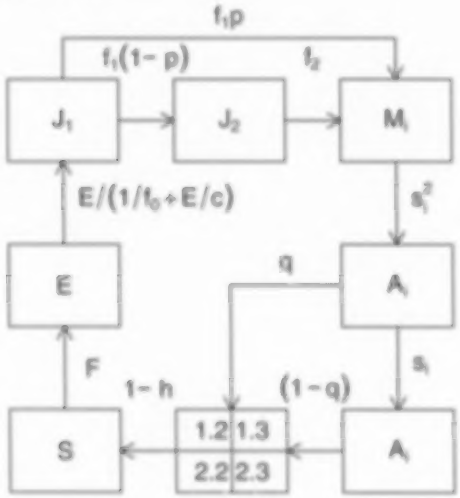
Fig. 2. Schematic diagram of life-history model stages. *S*, spawners; *E*, eggs; *J*, age 1 or 2 juveniles; M_i, age *i* smolts; A_i, ocean year *i* adults; 1.2, 1.3, 2.2, 2.3 are the "freshwater years.ocean years" ages of adults.



$$J_{a,y+2} = J_{a-1,y+1} f_{a,y+1} \tag{3}$$

The number of smolts migrating after 1 year in the lake is the number of 1-year-old juveniles times the proportion that migrate.

$$M_{1,y+2} = J_{1,y+2}\, p_{y+2} \tag{4}$$

The number of 2-freshwater smolts migrating is the number that do not migrate as 1-freshwater smolts.

$$M_{2,y+3} = J_{1,y+2}(1 - p_{y+2}) f_{2,y+2} \tag{5}$$

The adult return of 1.2s is the number of 1-freshwater smolts times the survival of 1-freshwater smolts times the proportion of smolts that return after 2 years in the ocean.

$$A_{1.2,y+4} = M_{1,y+2}\, q_{y+2}\, s^2_{1,y+2} \tag{6}$$

A simplified representation of survival is implemented to reduce the number of estimated parameters. Only one ocean survival rate is used for each freshwater age across all the year in the ocean. The survival rate thus overestimates the survival in the first year when fish are most vulnerable and underestimates the survival rate in later years in the ocean when when survival would typically be higher. The adult return of 1.3s is the number of 1-freshwater smolts times the survival of 1-freshwater smolts times one minus the proportion returning as 2-ocean fish.

$$A_{1.3,y+5} = M_{1,y+2}\,(1 - q_{y+2})\, s^3_{1,y+2} \tag{7}$$

The 166 adult return of 2.2s is the number of 2-freshwater smolts times the survival of 2-freshwater smolts times the proportion of smolts that return after 2 years in the ocean.

$$A_{2.2,y+5} = M_{2,y+3}\, q_{y+3}\, s^2_{2,y+3} \tag{8}$$

The adult return of 2.3s is the number of 2-freshwater smolts times the survival of 2-freshwater smolts times one minus the proportion of smolts that return after 2 years in the ocean.

$$A_{2.3,y+6} = M_{2,y+3}\,(1 - q_{y+3})\, s^2_{2,y+3} \tag{9}$$

Spawners are all the adults that made it past a fishery with exploitation rate *h*.

$$S_y = (1 - h) \sum_i \sum_j A_{i,j,y} \tag{10}$$

We assume that 2-freshwater smolts will have a higher ocean survival than 1-freshwater smolts. From these equations we predict the number of 1.2, 2.2, 1.3, and 2.3 adult returns. We assume that the likelihood of the observed return, given the predicted return, is normally distributed

$$\mathcal{L}(O_{i,j,y}|A_{i,j,y}) = \prod_{y=1}^{n} \prod_{i=1}^{m} \prod_{j=1}^{p} \frac{1}{\sigma\sqrt{2\pi}} \times \exp\left[-\frac{(O_{i,j,y} - A_{i,j,y})^2}{2\sigma^2}\right] \tag{11}$$

where the standard deviation σ^2 is estimated from the data. We minimize the negative log-likelihood for our parameter search, substituting the maximum likelihood estimate (MLE) for σ^2, $\hat{\sigma}^2 = \sum_y \sum_i \sum_j \frac{(O_{i,j,y} - A_{i,j,y})^2}{mnp}$. The likelihood term for smolts uses the same form as eq. 11, but uses observed and predicted smolt numbers for each smolt age instead of adult numbers. We use the software AD Model Builder (ADMB) Fournier (2001) to minimize the negative log-likelihood of the normally distributed observation error model in eq. 11 (adding the smolt likelihood in a separate analysis). It is an optimization algorithm that searches for parameter combinations that minimize an objective function. We penalize the parameter search by constraining certain parameters to fall within predetermined ranges. We constrain the 183 fecundity parameter to be centered on 3700 eggs per female with a coefficient of variation of 0.3. We further constrain the parameter search by estimating a scaling parameter γ such that $s_{1,y} = \gamma_y s_{2,y}$, where $\gamma_y \in (0, 1)$. This guarantees that $s_{1,y} < s_{2,y}$, based on the assumption that older smolts will have higher survival rate that younger (and presumably smaller) smolts. All survival rates and migration rates are constrained to the range (0, 1).

Residuals of Ricker stock–recruitment analyses of Bristol Bay stocks have shown lag-1 serial autocorrelation in residuals (Farley et al. 2006). For a comparison with LHM results obtained from eq. 11, we perform a model fit using the first-order autoregressive (AR(1)) error model $\varepsilon_t = \rho\varepsilon_{t-1} + \gamma_t$, where $\gamma_t \sim N(0, \sigma_\gamma)$. We use the fact that $\varepsilon_t - \rho\varepsilon_{t-1} = \gamma_t$ and $\sigma^2_\gamma = (1 - \rho^2)\sigma^2_\varepsilon$ to minimize the negative log of the likelihood in eq. 12.

$$\mathcal{L}(O_{i,j,y}|A_{i,j,y}) = \frac{1}{\sigma_\varepsilon\sqrt{2\pi}} \exp\left[-\frac{\varepsilon_1^2}{2\sigma^2_\varepsilon}\right] \prod_{y=2}^{n} \prod_{i=2}^{m} \prod_{j=2}^{p} \times \frac{1}{\sigma_\varepsilon\sqrt{(1-\rho^2)2\pi}} \exp\left[-\frac{\gamma_t^2}{2(1-\rho^2)\sigma^2_\varepsilon}\right] \tag{12}$$

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Model definition and selection

We define and compare models that have different levels of complexity. The BH stock–recruitment model aggregates the entire life history into a single stage from spawners to adults. The simplest LHM is the constant rate (CR) model, which expresses life-history stages distinctly, but without varying life-history parameters from year to year. The variable rate (VR) LHM also expresses each stage explicitly, but additionally assumes that survival rates vary year to year. With n years of data and four predicted age classes, there are $4n$ data points. When we assume that all survival rates vary year to year, we assume that $f_1 = \phi f_2$ and $s_1 = \varphi s_2$ (where $\phi \in (0, 1)$ and $\varphi \in (0, 1)$ are estimated). This adds $3n + 2$ parameters (n for each of f_0, f_2, and s_2 and 1 for each of ϕ and φ). The CR and VR models are compared using Akaike's information criterion for small samples (AIC_c) because the likelihoods used to evaluate them are based on the same observed quantities (Burnham and Anderson 2002), but the BH model cannot be compared with them because observations are aggregated. To include the results of fitting the BH model to the data in this investigation, we interpret the implications of the parameter estimates and uncertainty with respect to policy variables. Thus, the relative benefit of expressing the additional details of the VR vs. CR models are discussed along with their resulting policy variables as compared with the simple BH model. We compare the CR model with the VR model for cases with and without smolt data. Results are presented in comparison with the BH model and not the Ricker model, because the comparison reduces the ambiguity about whether results obtained from the LHM are a result of the functional form or the data aggregative effect.

Policy evaluation

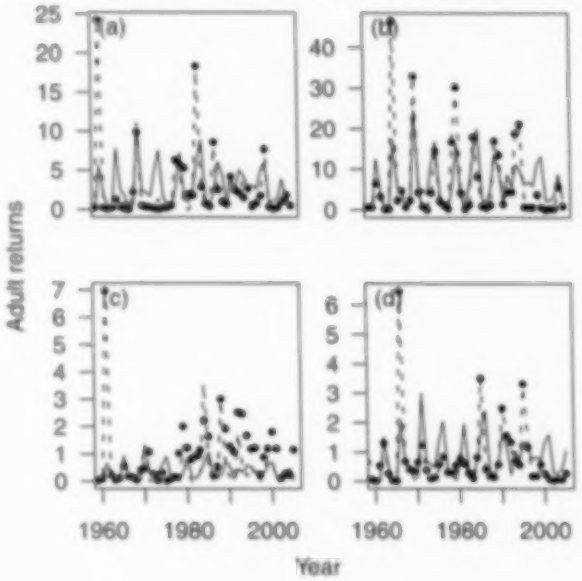
We calculate the optimal exploitation rate U_{MSY} and the optimal spawning stock size S_{MSY} with the LHM model by using estimated parameters to predict population trends from known historical starting conditions, such as 1956–1960 escapements. We use optimization routines written in ADMB to find spawning stock sizes and exploitation rates that would produce the highest average catch throughout a 200-year simulation ignoring the first and last 10 years. BH policy numbers are obtained by using the formulas $U_{MSY} = 1 - \sqrt{\frac{1}{a}}$, where $U_{MSY}S^*$ is the optimal yield calculated by solving $S^* = \frac{a(1-U_{MSY})S^*}{1+\beta(1-U_{MSY})S^*}$ in terms of $U_{MSY}S^*$. $S_{MSY} = b\sqrt{\frac{1}{a}} - \frac{b}{a}$ and it's yield is $R - S_{MSY}$. The parameters a and b come from the BH equation $R = aS/(1 + aS/b)$.

Model-fitting and uncertainty

Parameter estimates are the joint MLEs obtained by minimizing the negative log-likelihood of eq. 11. By using the small sample AIC_c to compare fits of different levels of model complexity, we select models that avoid overfitting the data (i.e., explaining noise rather than process). If we have n years of data, then we have $4n$ observations for the four major age groups occurring 4, 5, or 6 years after spawning. Our prediction is that observed returns having originated from different brood years contain information on survival rates at every stage. By virtue of the fact that the prediction of each data point is affected by seven to nine parameters (depending on migration timing), it can be very unambiguous what the survival rates needed to be to explain the observed trends.

We explore variability in parameter estimates of the CR model by saving Markov chain Monte Carlo (MCMC) samples from each parameter estimate (Brooks 1998) and plotting the MCMC posterior frequency distributions for each parameter. We used the built-in Metropolis–Hastings algorithm (Chib and Greenberg 1995) in ADMB, which uses the inverse of the Hessian matrix at the mode of the joint MLE to build a multivariate normal jumping distribution and rescales jumping rates to maintain approximately a 30% acceptance rate of posterior samples. We sampled every 1000th simulated parameter value from 2 000 000 MCMC simulations saved after burning-in the simulation and discarding 100 000 iterations. We saved 2000 values of the MCMC chain and used these to generate the frequency histograms.

Fig. 3. Fit of models the Kvichak adult age class data (in millions). Solid and broken lines are predicted adult returns from constant rate (CR) and variable rate (VR) models, respectively, and circles are observed data. Panels corresponds to adult returns for (freshwater years.ocean years) (*a*) 1.2, (*b*) 2.2, (*c*) 1.3, and (*d*) 2.3.



Results

The maximum likelihood fit of the CR and VR models to the Kvichak observed returns is displayed (Fig. 3). It shows the predicted adult returns of each age class. Fitting results are only shown here for the Kvichak system, mainly to illustrate the fact that each age class is fit separately. The estimation of parameters need not guarantee that all age classes fit the data equally well, but rather that the negative log-likelihood is minimized for a particular parameter combination. In particular, if one age class is less abundant than another, it will have less of an impact on the total likelihood

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(see Fig. 3c, where the abundance of 1.3s is far less than that for the 1.2s and 2.2s). For each system analyzed, there is overwhelming support (Table 2) for selecting the more parsimonious CR model. Despite using an additional 144 parameters to fit the 255 data, the improvement in the fit does not warrant the extra complexity.

Our primary concern in this investigation is to compare life-history models with simple spawner recruit models for the purpose of establishing S_{MSY}. No density-dependent pattern in recruits per spawners is evident (Fig. 1*b*). Ignoring for a moment the histograms in Fig. 4, we show the simple BH fits to each of the systems along with the resulting recruitment predictions when the LHM models (with and without smolt data) are employed. The life-history models clearly show more bending in the LHM curves (Figs. 4*a*–4*c*), which has the result of predicting lower values of S_{MSY}. The addition of the smolt data to the analysis does not detect much more density dependence in the Ugashik or Kvichak systems (see the relative positions of S_{MSY} in Figs. 4*a* and 4*c*), but results in roughly a 50% lower S_{MSY} prediction in the Egegik system (Fig. 4*b*).

We performed an uncertainty analysis on the CR models to examine the relative benefits of using smolt data. We show the estimated frequency distributions of parameters for the Ugashik system and contrast those distributions with and without smolt data (Fig. 5). There is clear evidence of a reduction in uncertainty in ocean survival, which is also true for the other systems, but we also see decreased uncertainty in f_0 and C. No substantial improvement in the precision of the estimation of f_1 and f_2 is evident with the inclusion of smolt data in any of the systems.

We repeated the model-fitting using the AR(1) error likelihood and found no appreciable difference in the MLEs of U_{MSY} and S_{MSY}, nor any appreciable reduction in variation in the posterior distributions of the life-history parameters. Though not tabulated, the magnitude of the differences in U_{MSY} and S_{MSY} estimates obtained using the AR(1) likelihood was small as compared with the magnitude of the differences between the LHM and the BH model.

Policy implications

Both types of LHM models consistently predict lower values of S_{MSY} than BH models (Fig. 4). This result is most pronounced in the Egegik and Kvichak systems (see Figs. 4*b* and 4*c*), where BH models predict S_{MSY} values at spawning stock sizes three or more times greater than observed historically. In contrast with BH predictions, the LHM models consistently predict S_{MSY} within the range of historically observed spawner numbers. The range of uncertainty in S_{MSY} from LHM models is shown (see histograms in Fig. 4). This was obtained by re-evaluating S_{MSY} for each saved parameter vector from MCMC simulations and plotting the relative frequencies of S_{MSY}. Two results stand out: (*i*) smolt data does not substantially change the mean value of S_{MSY} (see Figs. 4*a* and 4*c*, with the exception of the Egegik system in Fig. 4*b* showing 50% reduction); (*ii*) the distribution of S_{MSY} appears to be bimodal (see Figs. 4*a*–4*c*). Histograms in Fig. 4*b* appear off the scale of the figure, well beyond the range of the data and are truncated, but are still bimodal (see Fig. 6). A possible explanation for the bimodality seen here is that the data span a time frame over which the systems are widely speculated to have undergone regime shifts, in which case the modes would represent the policies under each regime.

Table 2. Summary of Akaike's information criterion for small samples (AIC_c) and associated statistics for constant rate (CR) and variable rate (VR) models.

System	Model	$-\log(\mathcal{L})$	K	AIC_c	Δ_i
Ugashik	CR	1435	9	2889	0
	VR	1303	153	4725	1836
Egegik	CR	1554	9	3127	0
	VR	1440	153	4998	1871
Kvichak	CR	1665	9	3349	0
	VR	1607	153	5332	1983
Wood	CR	1289	9	2597	0
	VR	1269	153	4657	2060

Note: Δ_i show the AIC_c of each model relative to the best fit (i.e., $\Delta_i = AIC_{c,i} - \min[AIC_c]$). $\Delta_i > 10$ implies essentially no support for that model.

The means and standard deviations of U_{MSY}, S_{MSY}, and their resulting yields (Table 3) evaluated from the MCMC samples do not necessarily correspond to the maximum likelihoods depicted in Fig. 4. Because BH models produced S_{MSY} estimates much higher than historical abundances in some cases, we sought to force the fitting procedure to estimate parameters that would yield S_{MSY} estimates within historical ranges. In an additional analysis (not shown), we assumed a Bayesian prior for the BH parameter b equal to approximately double the maximum number of returns historically observed. Priors on b parameters for the Ugashik, Egegik, and Kvichak systems would then be 6.6, 46, and 99.5 million, respectively. Adding priors to analysis predicts lower yields, but the requirement to do so casts doubt on the traditional BH method. The implied adult capacity values that arise from the estimated fry capacity parameter (C) from the LHM analysis were obtained by multiplying C values by the freshwater and ocean survival rates and obtaining a weighted average across all stages of life history. The weights were simply the proportions of each age class implied by the parameters p and q. Quinn (2005) summarizes various salmonid survival rates that are used to derive these capacities. Multiplying our estimated C values by the egg-to-smolt survival rate and smolt-to-adult rates in (Quinn 2005), we arrive at adult capacities of 11.7, 69, and 113 million, respectively, for the Ugashik, Egegik, and Kvichak systems. These are very comparable with the prior b values that were required to get reasonable S_{MSY} estimates from the BH models, further evidence that LHM capacity estimates appear to predict trends within empirically observed ranges.

Discussion

The most important result we find in this analysis is that the LHM approach detects more density dependence than conventional stock–recruitment methods. When the BH model is used, the density-dependent b parameter estimates are very high with large variance, U_{MSY} estimates are low, and MSY estimates are high as compared with historical abundances. To cause MSY estimates to be closer to historical values, an informative prior must be placed on the BH b parameter. We find that the LHM model estimates histori-

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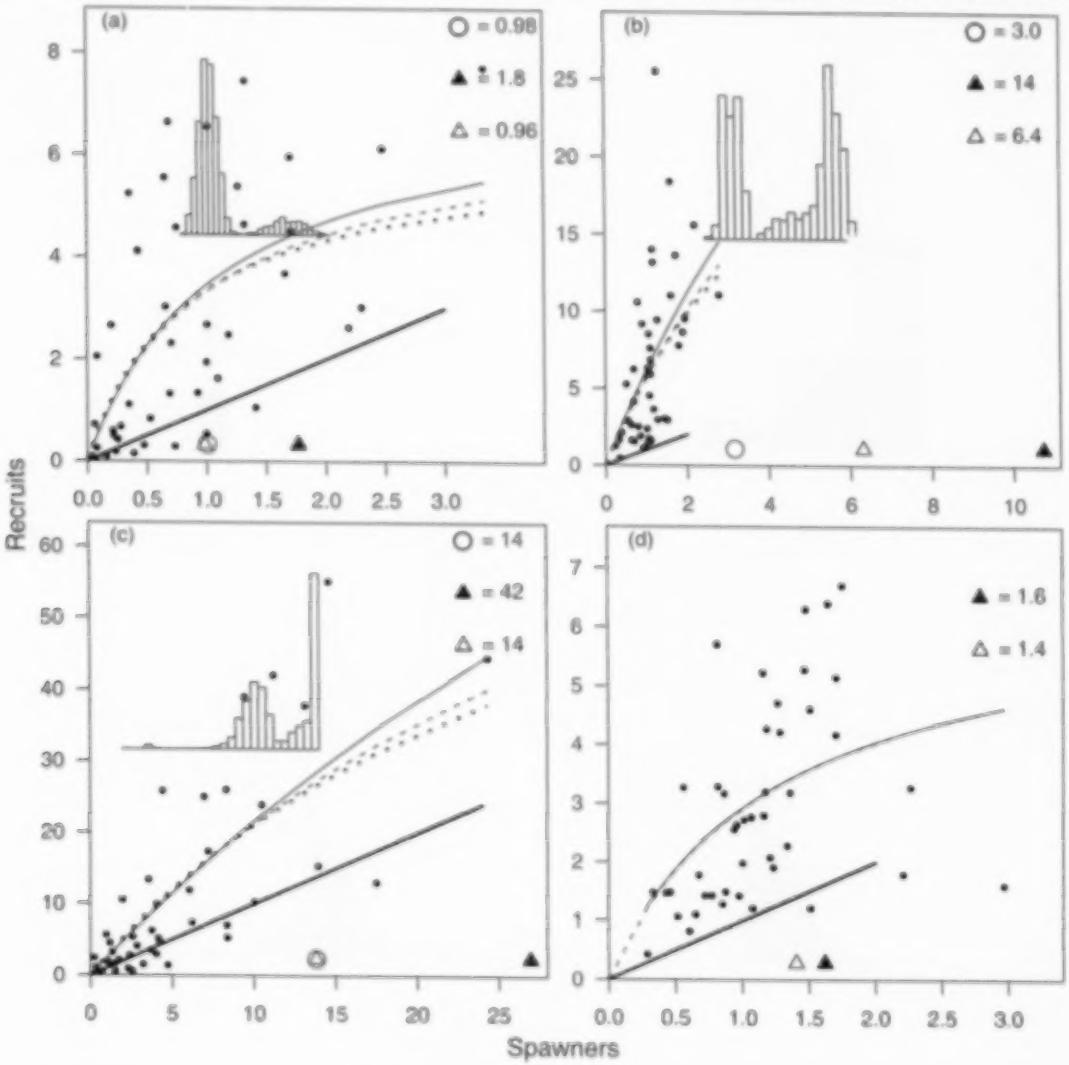
Fig. 4. Model fits to stock–recruitment data and estimated optimal spawning stock size (S_{MSY}) for optimal sustained yield derived from Beverton–Holt (BH) and constant rate (CR) models with and without smolt data. Data points are observed recruits of all age-class returns for a given spawner abundance. Solid lines are best fits of BH spawner recruit models. Dashed lines are best fits of the life-history model (LHM) without smolt data, and dotted lines are the LHM fit with smolt data (LHM_s). Thick solid lines show 1:1 replacement. Light grey triangles show S_{MSY} using the LHM model, clear circles show the result for LHM_s, and black triangles show maximum likelihood estimates of S_{MSY} using a simple BH model (shown at the *x*-axis limit when beyond scale of figure). Upper and lower histograms show ranges of uncertainty in S_{MSY} from Markov chain Monte Carlo (MCMC) simulations with and without smolt data smolt, respectively. Panels (*a*), (*b*), (*c*), and (*d*) correspond to the Ugashik, Egegik, Kvichak, and Wood (no LHM_s) systems, respectively. All number are in units of millions.



cally consistent MSY values for each system analyzed, but does so without imposing priors on the estimation of parameters. In part, our results in this analysis might be attributable to the fact that Bristol Bay sockeye populations have complex age structures and that density dependence is detectable as a result of observed trends having been subject to survival rates in different years. This outcome may not be true in cases of most pink and coho salmon, nor for several of the Fraser River sockeye stocks (i.e., stocks composed primarily of a single age class).

Density dependence, whether manifested as a maximum fry production capacity *C* in the LHM or in the BH *b* parameter, profoundly impacts harvest strategies and estimated MSY. The encapsulation of such an important determinant of system performance into a single parameter necessarily invites this problem, but by placing some restrictions on its value, a robust relationship between spawners and recruits can be achieved. Take, for example, the Kvichak system. The reason the estimate of *b* is so high is because some anomalous conditions occurred and forced the estimation

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Fig. 5. Estimated posterior probability distributions of parameter values for the Ugashik system, showing the relative frequency of parameter values sampled randomly from Markov chain Monte Carlo (MCMC) simulations: (*a*) fry production capacity in millions, (*b*) fecundity, (*c*) smolt migration rate, (*d*) spawning migration rate, (*e*) survival to fry stage, (*f*) survival first year in the lake, (*g*) survival second year in lake, (*h*) ocean survival of 1-freshwater smolts, and (*i*) ocean survival of 2-freshwater smolts. Shaded bars show the posteriors for the constant rate (CR) model. Transparent bars show the posteriors when smolt data is included in the CR model.

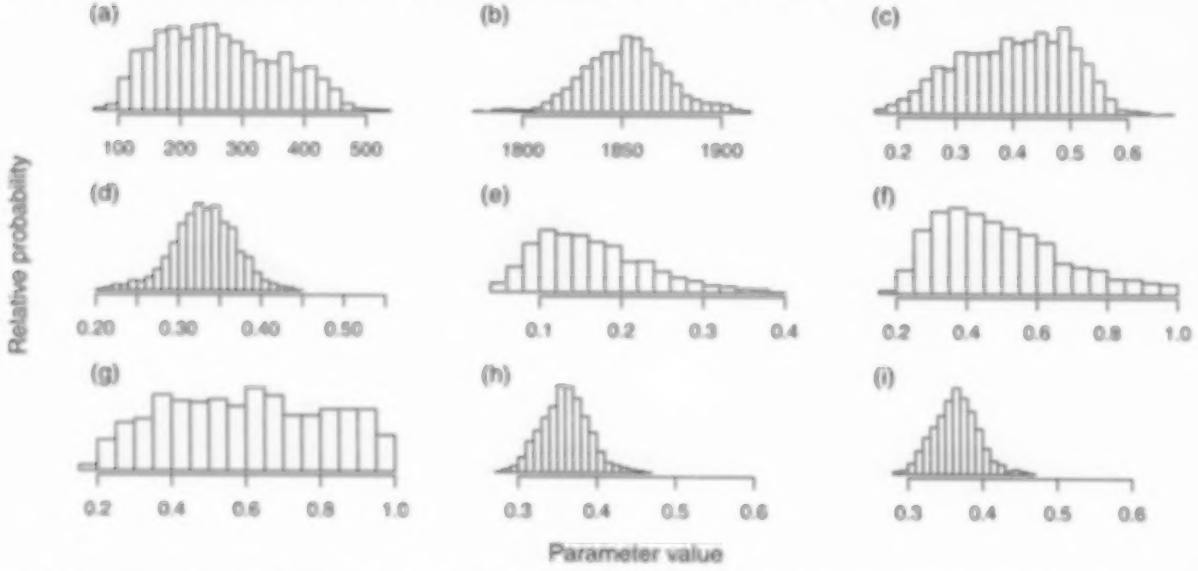
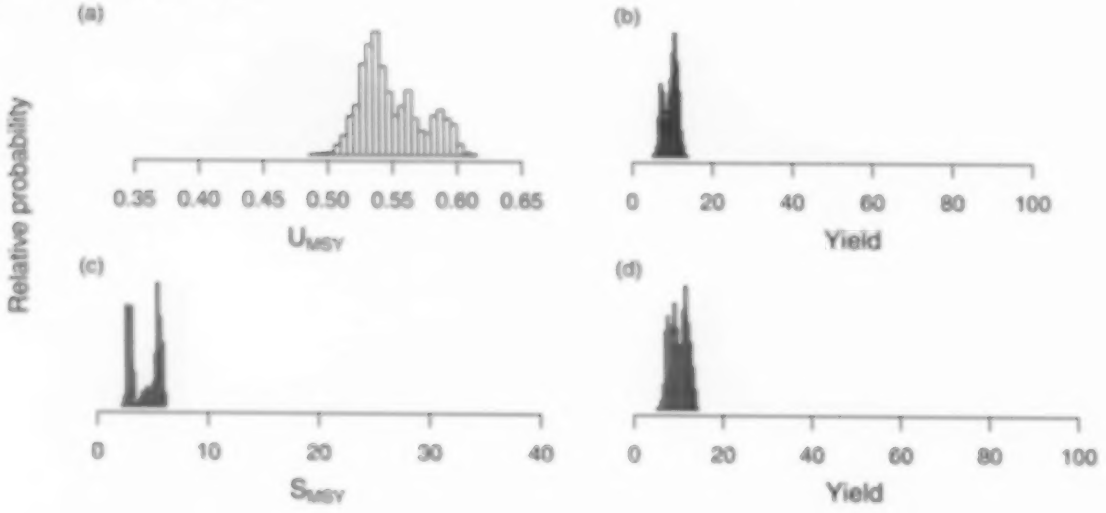


Fig. 6. Posterior distributions of optimal sustained exploitation rate (U_{MSY}), optimal sustained spawning stock size (S_{MSY}) in millions, and resulting yields in millions (right column) of the Egegik system. Shaded histograms show the analysis without smolt data; clear histograms show the result including smolt data.



routine to give weight to extremely productive but rare conditions. There were some very productive years in the early 1960s, where returns of 1.2s and 2.2s alone were around 60 million. The likelihood we used gave a lot of weight to parameter choices that could support such an occurrence, but predominantly, returns were less than half of this in peak years and an order of magnitude lower in off-cycle years.

The main problem with using the LHM model is that it predicts the number of fry produced, whereas we have limited knowledge of the number of fry that are emerging from streams. Nonetheless, scaling C estimates to adult numbers resulted in reasonable empirical values. In contrast, placing a penalty on the b parameter in the BH model is less ambiguous because b pertains directly to the number of returning adults, which are an observed quantity. Placing Bayesian

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Table 3. Mean optimal exploitation rate for maximum sustained yield (U_{MSY}), optimal sustained spawning stock size (S_{MSY}), and resulting yield (in millions) obtained from saved Markov chain Monte Carlo (MCMC) simulated parameters.

System	Model	U_{MSY}	Yield	S_{MSY}	Yield
Ugashik	LHM	0.56 (0.05)	2.1 (0.25)	1.0 (0.22)	2.2 (0.27)
	LHM$_s$	0.54 (0.05)	2.0 (0.27)	1.1 (0.27)	2.1 (0.3)
	BH	0.57 (0.1)	8.4 (10)	3.9 (4.7)	4 (2.8)
Egegik	LHM	0.49 (0.05)	22.8 (13.2)	8.5 (6.6)	21.1 (13.6)
	LHM$_s$	0.55 (0.02)	9.7 (1.7)	4.3 (1.3)	10.1 (1.9)
	BH	0.63 (0.04)	29 (13)	13 (6)	21 (8.5)
Kvichak	LHM	0.21 (0.03)	16.0 (7.6)	14.5 (3.1)	10.9 (3.4)
	LHM$_s$	0.31 (0.01)	8.2 (0.78)	11.4 (2.1)	8.8 (1.2)
	BH	0.34 (0.04)	266 (157)	102 (57)	50 (27)
Wood	LHM	0.59 (0.04)	1.7 (0.17)	1.3 (0.22)	1.7 (0.18)
	BH	0.56 (0.09)	4.9 (5.1)	2.3 (2.2)	2.5 (1.1)

Note: Standard deviations are shown in parentheses. LHM, life-history model (LHM) without smolt data; LHM$_s$, life-history model with smolt data; BH, Beverton–Holt.

priors on the b parameters in the BH model lowered predicted S_{MSY} values, but in some cases this was the only way to bring them to empirically consistent values. A possible extension to the LHM is to add a relationship between freshwater survival rates and the size of juveniles. Since size data is routinely collected, this could improve estimates of C (a critical parameter in determining S_{MSY}) by unconfounding juvenile survival and C estimation, which would provide additional insight into the early life history of sockeye populations.

Our AIC analysis suggested that we should not use the VR models, but additional insights could be gained by analyzing models that use variable rates in relation to time series of environmental variables by creating functional relationships between survival and environmental variables such as average lake temperature and sea surface temperature. This would avoid estimation of n parameters for each rate, instead estimating just a few parameters to establish the functional relationship and letting the data determine the value of the demographic rate. Another avenue worth exploring would be to study the interactions between stocks. There is some indication from the effect of smolt data on the resulting S_{MSY} values of the Kvichak and Egegik systems that adult return data alone can bias perceived productivity if there are interactions between the fisheries that either result in interceptions of nontarget fish or in catches being allocated to the wrong fishery, thereby affecting the data. This result is consistent with the actions of Bristol Bay managers in the 1990s, where fishing regulations in the Egegik district were implemented to prevent interceptions of Kvichak fish in the Egegik fishery. Explicitly modelling such interactions into the LHM could be useful to directly test hypotheses of such interactions. It has the additional benefit of being able to model stocks so that marine survival rates do not differ among stocks. This would force parameters to satisfy not only one populations trend, but also the trends of one stock with which it is hypothesized to share fishery and natural life-history characteristics.

We conclude that the LHM method predicts optimal spawning stock sizes more consistent with historical trends than escapement goal polices obtained from traditional stock–recruitment methods. This contrast is seen even with the limited data available in this analysis. The method shows that the density-dependent signal is lost during the aggregation of age-class returns for traditional stock–recruitment analysis, and this information loss can be misleading to policy and result in lost yield. The results suggest that the LHM method is useful for reconstructing the life histories of semelparous fish stocks that have complex age structure, but we have no evidence that this would be the case for stocks with simple age structures. However, even with simple age structure, the LHM approach still provides the opportunity to fit models to data sources available at intermediate life-history stages.

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List of symbols

E_y eggs deposited in year y
S_y number of spawners (males and females) in year y
F eggs per spawner
$f_{0,y}$ maximum egg-to-fry survival
C maximum fry production
$f_{a,y}$ survival rate in fresh water at age a in year y
$M_{i,y}$ number of i-freshwater smolts migrating in year y
p_y proportion of juveniles smolting at age 1 in year y
q_y proportion of 2-ocean adults spawning in year y
$s_{i,y}$ ocean survival of i-freshwater smolts in year y
γ_y constant, such that $s_{1,y} = \gamma_y s_{2,y}$, $\gamma_y \in (0, 1)$
$A_{i,j,y}$ predicted i-freshwater, j-ocean returns in year y
$O_{i,j,y}$ observed i-freshwater, j-ocean returns in year y
h harvest rate

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Overcompensatory response of a smallmouth bass (*Micropterus dolomieu*) population to harvest: release from competition?

Elise F. Zipkin, Patrick J. Sullivan, Evan G. Cooch, Clifford E. Kraft, Brian J. Shuter, and Brian C. Weidel

Abstract: An intensive seven-year removal of adult, juvenile, and young-of-the-year smallmouth bass (*Micropterus dolomieu*) from a north temperate lake (Little Moose Lake, New York, USA) resulted in an increase in overall population abundance, primarily due to increased abundance of immature individuals. We developed a density-dependent, stage-structured model to examine conditions under which population control through harvest could result in the increase of a targeted species. Parameter values were derived from a 54-year data set collected from another north temperate lake (Lake Opeongo, Ontario, Canada) smallmouth bass population. Sensitivity analyses identified the demographic conditions that could lead to increased abundance in response to harvest. An increase in population abundance with harvest was most likely to occur when either (*i*) per capita recruitment at low levels of spawner abundance was large, juvenile survivorship was high, and maturation of age-4 and older juveniles was moderately high or (*ii*) per capita recruitment at low levels of spawner abundance was slightly lower, yet the maturation rate of age-3 juveniles and adult survivorship were high. Our modeling results together with empirical evidence further demonstrate the importance of overcompensation as a substantial factor to consider in efforts to regulate population abundance through harvest.

Résumé : Des captures intensives pendant sept ans des adultes, des juvéniles et des jeunes de l'année d'achigans à petite bouche (*Micropterus dolomieu*) dans un lac de la région tempérée nord (lac Little Moose, New York, É.-U.) ont eu pour effet un accroissement dans l'abondance globale de la population, principalement à cause d'une augmentation de l'abondance des individus immatures. Nous avons élaboré un modèle dépendant de la densité et structuré en fonction des stades afin d'évaluer les conditions sous lesquelles un contrôle de la population par la récolte peut entrainer une augmentation de l'espèce ciblée. Les valeurs des paramètres ont été tirées d'une banque de données couvrant 54 années et provenant d'une population d'achigans à petite bouche d'un autre lac de la région tempérée nord (lac Opeongo, Ontario, Canada). Des analyses de sensibilité ont permis d'identifier les conditions démographiques qui pourraient mener à une abondance accrue en réaction à la récolte. Une augmentation de l'abondance de la population en réaction à la récolte va plus vraisemblablement se produire quand ou bien (*i*) le recrutement par individu aux faibles densités de reproducteurs est important, la survie des juvéniles est élevée et la maturation des juvéniles d'âge 4 ou plus est modérément élevée ou alors (*ii*) le recrutement par individu aux faibles densités de reproducteurs est un peu plus faible, mais malgré tout le taux de maturation des juvéniles d'âge 3 et la survie des adultes sont élevés. Les résultats de notre modélisation combinés à des données empiriques démontrent de plus l'importance de la surcompensation comme facteur important à considérer lorsqu'on tente de contrôler l'abondance d'une population par des récoltes.

[Traduit par la Rédaction]

Introduction

Optimizing resource use and maximizing productivity are common management objectives of harvest, but reducing abundance levels of undesirable species is another objective less frequently considered. Although harvest can be an effective method to control the abundance of nuisance or exotic species (Brooks and Lebreton 2001; Hein et al. 2006), control through harvest is complicated by factors such as demographic structure and density dependence. Individuals of different ages, sizes, or stages contribute disproportionately to future demography of populations due to disparities in survivorship and fecundity. Hauser et al. (2006) demonstrated that in a structured population, it is

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necessary to consider the relative reproductive value of each stage when devising a control harvest strategy. Additionally, the effects of a compensatory mechanism (i.e., density-dependent birth rates, growth rates, or mortality) can complicate efforts to control organisms through harvest (Brooks 2002). Recent studies have focused on optimal strategies to control overabundant populations and have demonstrated the potential for harvest to achieve this objective (Jensen 2000; Frederiksen et al. 2001; Hauser et al. 2007).

However, harvest can lead to unexpected dynamics such as increases in abundance of targeted populations, as has been reported for both plant (Paige 1992; Buckley et al. 2001; Newingham and Callaway 2006) and insect (Nicholson 1957; Cameron and Benton 2004) populations. Within a fisheries context, Matsuda and Abrams (2004) and Abrams and Matsuda (2005) demonstrated the potential for a predator population to increase in abundance with high mortality (such as that caused by harvest), which they referred to as the "hydra effect". Using theoretical models, their studies demonstrated that harvesting an unstructured predator population can either stabilize or destabilize a population, resulting in either an increase or decrease in population size, a response that varied according to the number of prey species, the interactions of the prey, and the functional response of the predator (Abrams and Vos 2003; Matsuda and Abrams 2004; Abrams and Quince 2005). However, empirical examples of this phenomenon have rarely been described in the fisheries literature (for a discussion of this topic, see Abrams and Matsuda 2005).

In this paper, we link harvest theory to an empirical study of an experimentally overexploited smallmouth bass (*Micropterus dolomieu*) population that has increased in abundance despite — and in response to — seven years of intense harvest. The removal effort has been successful in reducing the overall biomass of bass, primarily through a reduction of adults (>200 mm), subsequently leading to major increases in the abundance of six native fishes (Weidel et al. 2007) and increased piscivory and growth of the lake's apex fish predator, lake trout (*Salvelinus namaycush*) (Lepak et al. 2006). Yet despite these changes in food web linkages within the study lake, the relative abundances of juvenile bass (<200 mm) have increased throughout the time period of removal (Weidel et al. 2007). Although the overall biomass of smallmouth bass was reduced through the removal, the increase in juveniles has resulted in greater numbers of bass in the lake. This change in demography has important consequences for the continuation of the removal, namely that it may be necessary to continue the intense removal indefinitely to preserve the positive changes observed to date.

We developed a density-dependent stage-structured model to explore the response of the bass population to harvest with two key questions in mind. First, what are the demographic factors that could have led the population to respond to an intensive harvest with an overcompensation in abundance, specifically in young-of-the-year and juveniles? Second, what alternative harvest strategy could be employed to reduce the overcompensatory response of juvenile stages while simultaneously minimizing adult abundance? When determining whether harvest is a viable control option, it is essential to understand the circumstances under which demographic factors can confound control efforts. The model was developed to identify specific life stage factors that may be responsible for the apparent increase in overall abundance of the smallmouth bass population in response to an intense harvest.

Materials and methods

Smallmouth bass in Little Moose Lake

The study population is located in Little Moose Lake (271 ha), an oligotrophic lake in the Adirondack Park of northern New York State (Weidel et al. 2007). Smallmouth bass are not native to the Adirondacks or to many other north temperate lakes where introduced populations have led to declines in native fish abundance (Vander Zanden et al. 1999; Brown et al. 2000; Jackson 2002). As such, smallmouth bass population control is an important management objective for preserving native fish communities in north temperate lakes (Whittier and Kincaid 1999; Vander Zanden et al. 2004). Since their invasion into Little Moose Lake in the late 1940s, smallmouth bass have successfully dominated the littoral fish community in the lake (Brown et al. 2000). To improve conditions for native fishes, a smallmouth bass removal program was initiated in the spring of 2000 using targeted shoreline boat electrofishing. Prior to the removal, bass were surveyed in the springs of 1998 and 1999 to establish baseline levels of relative abundance and preremoval length distributions. From 2000 to 2007, smallmouth bass were collected and removed over the course of several weeks during both the spring and fall. A total of 53 947 smallmouth bass were removed over this time period. Details of the removal and methods are presented in Weidel et al. (2007).

We used electrofishing catch per unit effort (CPUE) data from standard nighttime surveys to examine how the relative abundance of the population has changed over the course of the removal. Although total smallmouth bass biomass has declined (Weidel et al. 2007), CPUE in numbers caught has increased from 1998 to 2007 (Fig. 1). Subdividing the population into length categories illustrates that although the abundance of adults (>200 mm) has declined, the relative abundances of young-of-the-year and yearlings (<100 mm) and older juveniles (100–200 mm) have increased since the onset of the removal (Fig. 2). The intensity of the harvest allowed us to estimate the minimum population abundance at the initiation of the harvest using a cohort analysis applied to the full set of cohorts present in 2000, followed through subsequent years using cohort slicing (Quinn and Deriso 1999). It was not feasible to use a cohort analysis to estimate the total abundance of the population at other times during the removal because it is not possible to accurately determine age from length data for smallmouth bass.

Model development

We developed a population model, using discrete time difference equations, with an annual time step during which smallmouth bass reproduce and are harvested once each year. The smallmouth bass population (N) was divided into four stages: yearlings (Y), age-2 juveniles ($J2$), age-3 and older juveniles ($J3$), and adults (A) (Fig. 3). Juveniles were separated into different stages because survivorship and fe-

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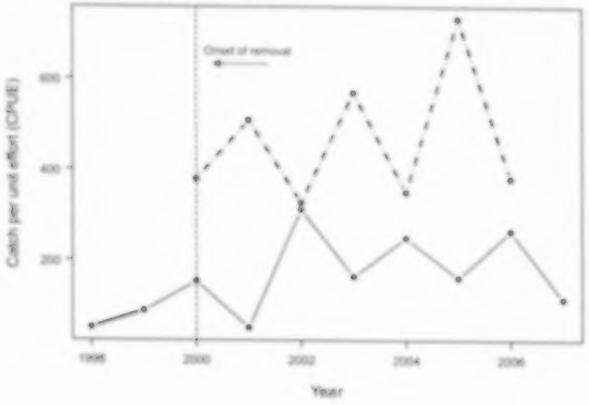
Fig. 1. Catch-per-unit-effort (CPUE) in number of smallmouth bass individuals captured (and removed starting in 2000) from Little Moose Lake (per hour of electrofishing run time) from 1998 to 2007. The solid and broken lines show results from spring and fall sampling periods, respectively.

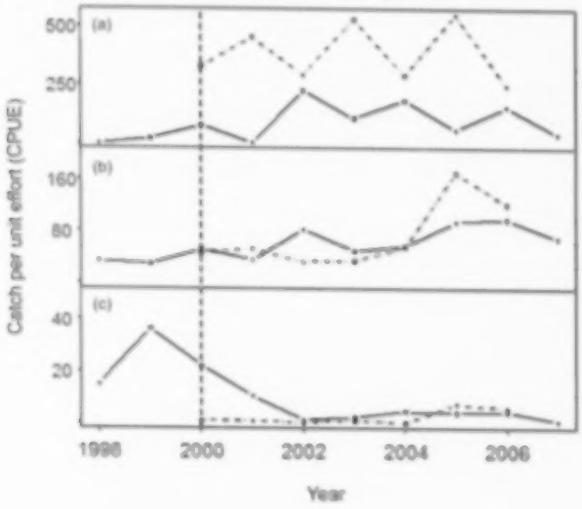


cundity vary as juveniles age (Rose 2005). The annual model cycle assumed that individuals are harvested immediately prior to breeding. We assumed that harvest occurs before breeding because (*i*) the annual spring removal of smallmouth bass is designed to occur prior to breeding in Little Moose Lake and (*ii*) this strategy minimizes the effort necessary for the removal (Doubleday 1975). Natural mortality was assumed to occur subsequent to breeding, followed by maturation to the next stage at the end of the time step. The model counts individuals prior to harvest (i.e., a prebreeding census each spring), which implies that yearlings at time t (Y_t) are age-1 and $J2_t$ juveniles are age-2. Individuals in stages $J3$ (immature) and A (mature) at time t are age-3 and older.

Given our interest in examining how stage-specific harvest affects smallmouth bass population dynamics, we assumed that harvest strategies could be varied to selectively remove differing proportions of each stage. We divided the harvest into three categories: an age-1 harvest (h_y), a harvest of age-2 and older juveniles (h_j), and a harvest of adult fish (h_a). The h_y harvest is applied to age-1 individuals immediately following their first birthday. We assumed that harvest does not occur during the first year of life so that individuals can first be harvested between age-1 and age-2. We combined the harvest of all age-2 and older juveniles, denoted by h_j, because it is impractical to distinguish these two categories in the field. The final harvest category is adult harvest, h_a, which is applied to mature fish. For simplicity, we assumed that harvest strategies remain fixed through time.

Characterizing the stock–recruitment relationship between the number of mature individuals and the number of offspring they produce is a long-standing challenge in the study of fish population dynamics (Ricker 1954; Beverton and Holt 1957; Myers 2001). Available evidence suggests that recruitment to age-1 for smallmouth bass may be overcompensatory (DeAngelis et al. 1991, 1993; Dong and DeAngelis 1998). An overcompensatory density-dependent relationship implies that total recruitment is maximized at an intermediate stock

Fig. 2. Annual catch-per-unit-effort (CPUE) of (*a*) young-of-the-year (in fall) and yearlings (in spring, <100 mm), (*b*) juveniles (100–200 mm), and (*c*) adults (>200 mm). The solid and broken lines show results from spring and fall sampling periods, respectively. The vertical broken line indicates the onset of the removal. Note the differences in scale in the three graphs.



level, beyond which it declines (Quinn and Deriso 1999). We used the Ricker (1954) stock–recruitment function for the yearling stage. The number of yearlings (Y) produced in $t + 1$ is dependent on the number of adults in time t that are not harvested ($1 - h_a$):

$$(1) \qquad Y_{t+1} = A_t(1 - h_a)\alpha e^{-\beta A_t(1-h_a)}$$

where $\alpha > 0$ and $\beta > 0$ are standard Ricker parameters regulating the maximum number of offspring produced per spawner and the magnitude of the density-dependent effects.

We assumed that natural mortality remains constant from year to year for all other stages. Yearlings (Y) that survive natural mortality (s_y; survivorship from age-1 to age-2) and are not harvested ($1 - h_y$) advance to the $J2$ stage:

$$(2) \qquad J2_{t+1} = Y_t(1 - h_y)s_y$$

$J2$ individuals that are not harvested ($1 - h_j$) survive at a rate of s_{j2} (survivorship from age-2 to age-3). They either remain as juveniles ($1 - m_1$), advancing into the $J3$ stage, or mature into adulthood at a fixed proportion m_1 (maturation rate of individuals at age-3). The proportion of $J3$ juveniles that survive annually is s_{j3} (annual survivorship of all immature individuals age-3 and older). $J3$ individuals that survive natural and harvest mortality can either remain as juveniles ($1 - m_2$) or mature into adulthood at a rate of m_2 (maturation rate of immature bass age-4 and older). We assumed that the maturation rates are constant so that a fixed proportion of both $J2$ and $J3$ individuals become adults on an annual basis. The equation for older juveniles is as follows:

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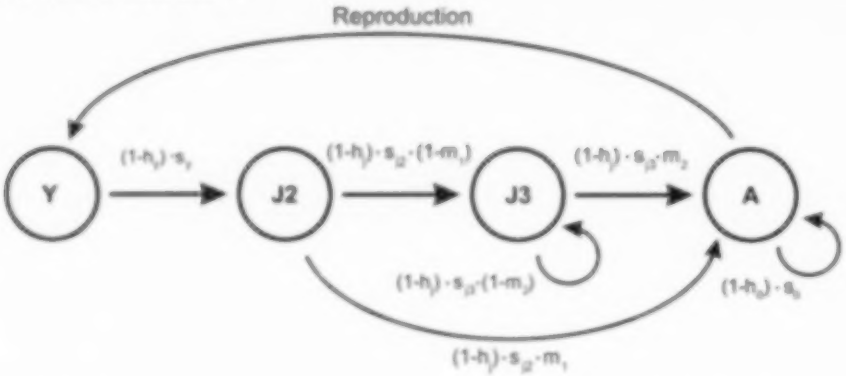
Fig. 3. Life cycle diagram for the smallmouth bass population model. The population is divided into four stages: yearling (*Y*), age-2 juveniles (*J2*), age-3 and older juveniles (*J3*), and adults (*A*). The model counts individuals prior to harvest with a prebreeding census each spring. Survivorship, *s*; harvest, *h*; maturation, *m*.



$$J3_{t+1} = J2_t(1-h_j)s_{j2}(1-m_1) + J3_t(1-h_j)s_{j3}(1-m_2) \quad (3)$$

The adult stage (*A*) in time $t + 1$ includes both the *J2* and *J3* juveniles from time *t* that survived harvest and natural mortality and matured into adulthood, as well as the adults from time *t* that survive natural mortality, s_a, and were not harvested:

$$A_{t+1} = J2_t(1-h_j)s_{j2}m_1 + J3_t(1-h_j)s_{j3}m_2 + A_t(1-h_a)s_a \quad (4)$$

Model parameterization

Because of the short length of the time series of data from Little Moose Lake and the intensity of the bass harvest, these data alone were insufficient for estimating the model parameters. Parameter values for the model were instead estimated using data from a smallmouth bass population in Lake Opeongo, a large (5860 ha), oligotrophic lake in Ontario, Canada. Smallmouth bass population information was collected continuously from 1937 through 1990 via creel surveys. Details of the Lake Opeongo data collection process and analysis are presented in Shuter et al. (1987).

We used annual approximations of age-specific abundances and estimates of the proportion of mature individuals at each age (averaged over the time series) to determine values for the parameters in the model. Values for survivorship and maturation were established by averaging annual rates over the 54 years of data collection. The values for s_y (survivorship from age-1 to age-2), s_{j2} (survivorship from age-2 to age-3), s_{j3} (average survivorship for older juveniles), and m_1 (maturation rate of age-3 individuals) were taken from Rose (2005), an analysis based on the work of Shuter et al. (1987) in which annual age-specific survivorship for smallmouth bass in Lake Opeongo was averaged over the time series. To determine the annual values for s_a (adult survivorship) and m_2 (maturation of age-4 and older individuals), we first grouped the Lake Opeongo data by our model stages (yearlings, age-2 juveniles, age-3 and older juveniles, and adults) to determine the total number of individuals in each stage in each year. Using the yearly survivorship values and the stage-specific population estimates, we solved eq. 3 for m_2 (assuming that there was no harvest in the population) to estimate annual values and then averaged those estimates to achieve a single parameter value for m_2. We repeated this process for s_a using eq. 4. The Lake Opeongo smallmouth bass abundance estimates included mortality from recreational fishing; any recreational fishing mortality was therefore incorporated into the model as natural mortality.

To parameterize the stock–recruitment relationship, we related the number of mature individuals (*A*) in year *t* to the number of one-year-olds (*Y*) in year $t + 1$ using the Ricker recruitment function. The parameters α and β (see eq. 1) were determined using a nonlinear minimization technique. Additional information on the stock–recruitment relationship in Lake Opeongo can be found in Shuter and Ridgway (2002). The parameter estimates generated from the Lake Opeongo smallmouth bass population are presented in Table 1.

Sensitivity analyses

Our primary interest was to evaluate circumstances under which harvest of one or more stages in a density-dependent population could lead to an increase in overall abundance. To this end, we evaluated the sensitivity of the projected dynamics from our model using two different approaches — an analytical approach and a simulation-based approach; the methodology for the two approaches is discussed below. In both cases, we reduced the parameter state–space (i.e., we nondimensionalized the model) by setting $\beta = 1$, without loss of generality.

Analytical approach

The objective of the analytical approach was to derive the condition under which harvest results in an increase in the total equilibrium population abundance, $\bar{N}$. The advantage of this approach was that we could explore the equilibrium solution over the entire parameter space. For simplicity and because the current harvest approach consists of removing all captured bass, we considered only the strategy in which all stages are harvested in equal proportions, $h_y = h_j = h_a$, which we denote by *h*.

The first step was to solve for the equilibrium population abundance for each stage in the model (i.e., we set $Y_t = Y_{t+1}$

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Table 1. Parameter values estimated from the Lake Opeongo smallmouth bass (*Micropterus dolomieu*) population.

Parameter	Description	Value
α	Ricker parameter — maximum per capita recruitment (without density dependence)	5.503055
β	Ricker parameter — magnitude of density dependence	0.000225
s_y	Proportion of *Y* that survive (natural mortality) to *J2*	0.74
s_{j2}	Proportion of *J2* that survive annually	0.74
s_{j3}	Proportion of *J3* that survive annually	0.61
m_1	Proportion of *J2* that mature into *A*	0.0560
m_2	Proportion of *J3* that mature into *A*	0.3725
s_a	Proportion of *A* that survive annually	0.54

and repeat for each stage). The total equilibrium population is the sum of the stages:

$$\widehat{N} = \widehat{Y} + \widehat{J}1 + \widehat{J}2 + \widehat{A} \quad (5)$$

The interest lies in how the equilibrium population $\widehat{N}$ is affected by a change in harvest. We took the derivative of $\widehat{N}$ with respect to harvest and evaluated the term at $h = 0$. If

$$\left.\frac{\partial \widehat{N}}{\partial h}\right|_{h=0} > 0 \quad (6)$$

then the slope of the equilibrium condition at the origin is increasing with respect to a positive change in harvest. This implies that there exists an $\widehat{N}$ that is greater in the presence of harvest ($h > 0$) than in its absence ($h = 0$). If eq. 6 does not hold, then it is not possible for the population to increase in response to harvest (i.e., the population cannot decrease with small levels of harvest but increase with large levels). The magnitude of the derivative (when positive) provides some information about the harvest intensity that will lead to a population decline (i.e., if the magnitude is small, then the population begins to decline quickly as h is increased).

The evaluation of the symbolic derivative of $\widehat{N}$ at $h = 0$ is not easily interpretable as the expression is complicated and seven parameters are included in the model ($\beta = 1$ in the nondimensional model). To better comprehend how various parameter combinations affect whether or not harvest leads to an increase in $\widehat{N}$, we repeatedly drew parameter estimates from a distribution of values and evaluated the sign of the derivative of $\widehat{N}$ at $h = 0$. We assumed that each of the survivorship and maturation parameters were beta-distributed with means equivalent to the values in Table 1. It was not possible to determine the potential variation around the survivorship parameter estimates because the yearly stage-specific abundances were frequently estimated through back calculations (Shuter et al. 1987). We set the standard deviations on the survivorship and maturation parameters reasonably high, from 0.05 to 0.09, to encapsulate potential variability. For each of these parameters, the distribution was dome-shaped with a peak at its estimated mean.

We reviewed previous studies that synthesized the spawner–recruit relationship across many species (Myers et al. 1999; Myers 2001; MacKenzie et al. 2003) to determine a potential range for the parameter α, the maximum number of recruits per spawner at low population densities. (None of the studies included estimates of a stock–recruitment relationship for smallmouth bass.) In 57 species examined by Myers et al. (1999), the annual number of spawners produced per spawner at low abundance (i.e., recruits that survive to maturity) ranged from 0.1 to 15.3 (with one outlier at 113.3), and the lifetime maximum reproductive rate (in spawners produced per spawner) ranged from 1.4 to 74.6 (same outlier at 123.5). Because we were interested in the number of age-1 individuals produced per spawner and not the number that survive to maturity, we examined a wide range for the parameter α ($1 \le \alpha \le 50$) to explore all potential dynamics in response to harvest.

To determine the population's sensitivity in response to harvest to changes in the parameter α, we evaluated the derivative of $\widehat{N}$ 10 000 times as each of the maturation and survivorship parameter values were randomly drawn from their potential distribution while holding α constant. We repeated this procedure for values of α from 1 to 50 by increments of 5. To determine sensitivities for the survivorship and maturation parameters, we varied each parameter individually through its potential range (from 0 to 1 by increments of 0.1) and evaluated the derivative of $\widehat{N}$ 10 000 times, where all other parameters were drawn from their unique beta distributions. In these cases, we again held α constant and repeated the process for several values of α.

Simulation-based approach

The numerical simulation-based sensitivity analysis was used to explore how changes in individual parameters affected whether the equilibrium population increased or decreased under various harvest strategies (with all other parameters held constant at their estimated values). The advantage of the simulation-based approach was that it allowed us to explore a larger variety of harvest strategies than was possible using the analytical approach alone. In addition, we used the numerical approach to determine the magnitude of the population increase in response to harvest (when it occurred) and the harvest level that maximized abundance.

We varied each of the individual parameters through their range of potential values while holding the remaining parameters constant at their estimated values. The survivorship and maturation parameters (s_y, s_{j2}, s_{j3}, s_a, m_1, m_2) have a potential range from 0 to 1 (i.e., 0%–100% survivorship and 0%–100% maturity) and were varied by increments of 0.1. The parameter α was again varied from 1 to 50 by increments of 5. We examined how individual parameter values affected the equilibrium population abundance under four harvest strategies. The scenarios were as follows: (*i*) harvest of age-1 bass only (h_1 varies, $h_2 = h_a = 0$); (*ii*) harvest of age-2 and older juvenile bass only (h_2 varies, $h_1 = h_a = 0$); (*iii*) harvest of adults only (h_a varies, $h_1 = h_2 = 0$); and

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(*iv*) harvest of all stages proportional to their relative abundance ($h_y = h_j = h_a$ and all vary). We chose to examine these four harvest strategies because they represent end points on the spectrum of potential approaches to harvest and combinations of these strategies produce results that are simple summations of each strategy on its own. Additionally, these harvest strategies can be practically applied in the field by comparison with strategies designed to remove specific proportions of each stage. For each of the harvest strategies, we varied every parameter individually through its potential range and observed whether or not the equilibrium population abundance was larger in the presence of harvest or in its absence, while holding all other parameter values constant.

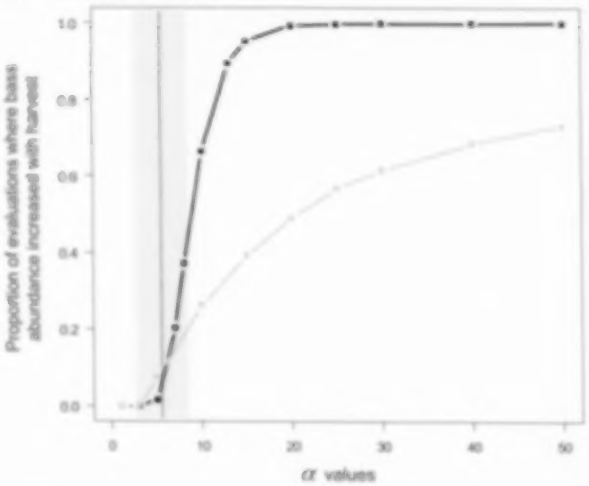
Results

Sensitivity analysis — analytical approach

The solution to eq. 6 confirmed that even though the yearling and juvenile stages could increase with harvest (which could lead to an increase in the abundance of $\hat{N}$), the adult population could not increase under a harvest strategy in which the stages were harvested in equal proportions, using any combination of parameter values. The results of the analytical sensitivity analysis demonstrated that the parameter α is key to determining the influence of harvest on smallmouth bass population dynamics. We evaluated the proportion of times out of 10 000 trials that the total equilibrium population $\hat{N}$ increased with harvest for values of α ranging from 1 to 50 when all other values were randomly selected from their unique beta distributions (Fig. 4). When α was between 1 and 3, the derivative of $\hat{N}$ at $h = 0$ was never positive and was positive in less than 2% of the trials for $\alpha = 5$. As the value of α increased, this percentage rapidly increased; at $\alpha = 15$, more than 95% of the simulations resulted in an increase in $\hat{N}$ with harvest. Because of the uncertainty surrounding the variance estimates on each of the survivorship and maturation parameters, we repeated simulations for the α values using a uniform distribution for all other parameters across their potential range (from 0 to 1). Under this naive scenario, we found the same trend in α; the proportion of simulations that resulted in an increase in $\hat{N}$ with harvest ranged from 0% to 73% as α was varied from 1 to 50 (Fig. 4).

Because of the large influence of the parameter α on the model dynamics, we examined the sensitivities of the survivorship and maturation parameters while holding α constant at several values. The maturation parameters were opposite in their sensitivities to harvest with respect to values of α (Fig. 5). When α was low ($\alpha \leq 5$), the response of the population to harvest was more sensitive to changes in m_1 than when α was high ($\alpha \geq 10$). For $\alpha = 5$, the value of m_1 had a large effect on whether harvest led to an increase in $\hat{N}$. As α was increased, harvest frequently led to an increase in $\hat{N}$ regardless of the value of m_1. The reverse was true for m_2. For $\alpha = 5$, harvest rarely resulted in an increase in $\hat{N}$, even as the value of m_2 approached 100%. For values of $\alpha \geq 10$, small increases in the value of m_2 above zero had a large effect on the response of the population to harvest. The population was similarly less sensitive to changes in the survivorship parameters s_y, s_j, and s_j, at $\alpha = 5$ compared with at $\alpha \geq 10$ (Fig. 5). The value of adult annual survivorship, s_a, was more important at values of $\alpha \leq 15$ and became less so as the value of α increased. At $\alpha = 5$, a relatively small change in value of s_a from 0.6 to 0.9 led the population to an increase in its equilibrium abundance with harvest from less than 1% of the evaluations to greater than 99% of the evaluations.

Fig. 4. Results of the analytical sensitivity analysis for the parameter α. The black line (with squares) shows the proportion of trials out of 10 000 evaluations that the derivative of the equilibrium population with respect to harvest was positive for each value of α (i.e., the population increased in response to harvest), assuming that the other parameters follow a beta distribution with mean values as in Table 1. The shaded line (with circles) shows the result under the naive scenario that all other parameters are uniformly distributed across their potential ranges. The vertical line and shaded area show the estimated value for α (Table 1) with a 95% confidence interval.



Sensitivity analysis — simulation-based approach

All survivorship and maturation parameters produced stable equilibriums throughout their ranges when each of the other parameters was held constant at its estimated value (in both the presence and absence of harvest). The stock–recruitment slope parameter, with values of $\alpha \geq 30$, produced cyclic levels of population abundance in the absence of harvest, although abundance stabilized when even small levels of harvest were applied. To avoid this cyclic behavior in our results, we limited the numerical sensitivity analysis to the range in which α produced stable equilibriums in the absence of harvest (i.e., $1 \leq \alpha \leq 25$). Running the model with the parameter values estimated from the Lake Opeongo data set did not result in an increase in population abundance with harvest. We used the results of the numerical sensitivity analysis to (*i*) demonstrate the extent to which each parameter would need to change (if all others were held constant) to produce an increase in population abundance with harvest and (*ii*) determine the magnitude of such increases when they occurred. The minimum value for each parameter (when all other parameters

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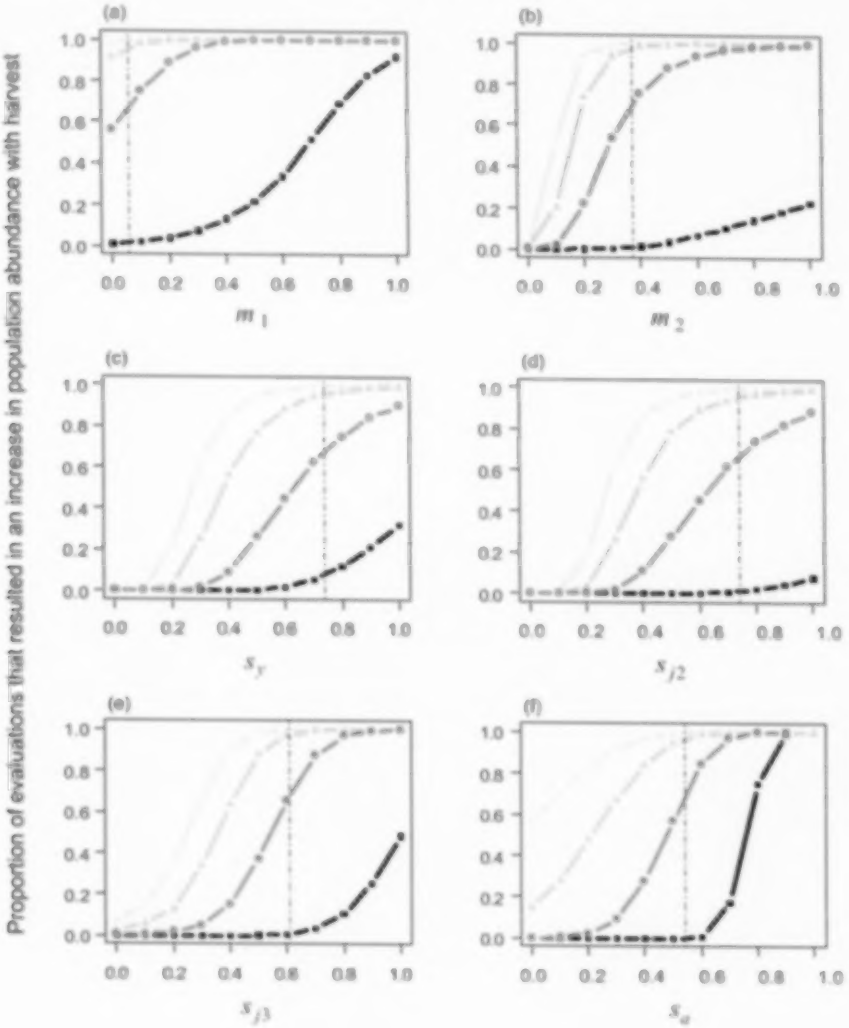
Fig. 5. Results of the analytical sensitivity analysis for (*a*–*b*) the maturation parameters (m_1, m_2) and (*c*–*f*) the survivorship parameters (s_y, s_{j2}, s_{j3}, s_a). The sign for eq. 6 is evaluated in 10 000 trials where each parameter is varied over its possible range (from 0 to 1 by increments of 0.1), and all other survivorship and maturation parameters are drawn from their unique distributions. Each panel shows the proportion of these trials that resulted in an increase in population abundance under harvest for α values of 5 (solid line with squares), 10 (dark shaded line with circles), 15 (light shaded line with triangles), and 20 (lightest shaded line with diamonds). The vertical broken lines show the estimated parameter value, using the smallmouth bass data from Lake Opeongo (Table 1).



were held at their estimated value) that would result in an increase in abundance under all four harvest strategies is presented in Table 2.

Increasing the value of the α parameter resulted in increases in the equilibrium population abundance $\tilde{N}$ of smallmouth bass under all harvest strategies except the yearling-only strategy. For $\alpha \geq 10$, the equilibrium population abundance was larger when all stages were harvested equally, when only age-2 and older juveniles were harvested, and when only adults were harvested. In those cases, the equilibrium abundances of both the yearling and juvenile stages also increased with harvest. Under the adults-only harvest strategy, $\alpha \geq 20$ also produced an increase in the equilibrium abundance of the adult stage. This was the only scenario in the sensitivity analyses in which an increase in the equilibrium abundance of the adult stage was observed with any type of harvest. When only the age-1 bass were targeted, $\alpha \geq 10$ led to an increase in the equilibrium of yearlings but not an increase in the total population $\tilde{N}$ (i.e., the increase in yearlings was less than the observed decreases in age-2 and older juveniles and adults, therefore total population size declined with harvest).

Increasing the maturation rate of *J2* individuals (m_1) or adult survivorship (s_a) also produced increases in the equili-

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Table 2. Results of the simulation-based sensitivity analysis.

	Total population harvest				Age-1 only harvest				Age-2+ juvenile only harvest				Adult only harvest			
Parameter	N	Y	$J2 + J3$	A	N	Y	$J2 + J3$	A	N	Y	$J2 + J3$	A	N	Y	$J2 + J3$	A
α	10	10	10	×	×	10	×	×	10	10	10	×	10	10	10	20
s_y	×	0.8	×	×	×	0.8	×	×	×	0.8	×	×	0.9	0.8	0.8	×
s_{j2}	×	0.8	×	×	×	0.8	×	×	×	0.8	×	×	0.9	0.8	0.8	×
s_{j3}	1.0	0.7	1.0	×	×	0.7	×	×	×	0.7	×	×	0.7	0.7	0.7	×
s_a	0.8	0.6	0.8	×	×	0.6	×	×	0.9	0.6	0.8	×	0.7	0.6	0.6	×
m_1	0.6	0.1	0.6	×	×	0.1	×	×	0.9	0.1	0.7	×	0.2	0.1	0.1	×
m_2	×	0.5	×	×	×	0.4	×	×	×	0.5	0.9	×	0.5	0.5	0.5	×

Note: Shown is the minimum value for each parameter that led to an increase in the equilibrium population abundance (and each of the stages) with all other parameters held constant at their estimated values under four harvest strategies: harvest of the total population, harvest of age-1 yearlings only, harvest of age-2 and older juveniles, and harvest of adults. An × indicates that the population (or stage) did not increase with any values of the parameter.

brium population abundance $\hat{N}$ (specifically in the yearling and age-2 and older juvenile stages), when only age-2 and older juveniles were harvested, adults alone were harvested, or each stage was harvested in equal proportions, but not when only age-1 individuals were harvested. The maturation rate of $J3$ individuals (m_2) and the survivorship parameters s_y (from age-1 to age-2), s_{j2} (from age-2 to age-3), and s_{j3} (survivorship of immature age-3 and older bass) were less influential. Increasing the value for each of these parameters led to an increase in $\hat{N}$ only with the adult-only harvest strategy.

In cases when harvest resulted in increased values of $\hat{N}$, the magnitude of the increases was highly variable (Table 3). Increasing the parameter α produced the largest increases in abundance for given harvest levels. At $\alpha = 25$, the population increased by as much as 25% (over the equilibrium abundance in the absence of harvest) when only age-2 and older juveniles were harvested and when equal proportions of each stage were harvested and 66% when only adults were harvested. Additionally, the adult-only harvest produced the largest increases in the total population abundance compared with the other harvest strategies.

Simulation results with Lake Opeongo parameter values

In the absence of harvest, the estimated equilibrium population size was stable at approximately 27 600 smallmouth bass ($Y = 9000$, $J1 = 6700$, $J2 = 7600$, and $A = 4300$) using the parameter values generated by the Lake Opeongo data set. The cohort analysis estimated that the Little Moose Lake bass abundance in the spring of 2000 (prior to the initiation of the removal) was approximately 7000 individuals, excluding age-0 fish. The Lake Opeongo bass population estimate was almost four times greater than the estimated population size in Little Moose Lake, which is not unexpected given that Lake Opeongo is much larger (5860 ha) than Little Moose Lake (271 ha). The carrying capacity for the model is determined by the parameter β (for given values of α). The nondimensional version of the model (where we set $\beta = 1$) demonstrated that β does not have an effect on the population dynamics, so this discrepancy in equilibrium population size did not influence our results.

Using the parameter values generated with the Lake Opeongo data set, the smallmouth bass population did not increase in abundance with harvest. Under all harvesting scenarios, the equilibrium population (and each of the stages) was reduced in the presence of harvest. The model population declined to zero with a total annual harvest of as little as 18% (where each stage is harvested in equal proportion). Alternatively, harvesting either 37% of age-2 and older juveniles ($h_j = 0.37$, $h_y = h_a = 0$) or 44% of the adults ($h_a = 0.44$, $h_y = h_j = 0$) also resulted in a population collapse.

The short-term population responses to harvest (Fig. 6) were similar to the long-term dynamics in that we never observed the population increasing in response to harvest with the Lake Opeongo parameters when simulations were initiated using the equilibrium population size and structure. This suggests that the observed increase in smallmouth bass abundance in Little Moose Lake may not simply reflect transient dynamics on a path to a consistently reduced population resulting from continual harvest. Furthermore, altering the annual harvest in the model (so that each harvest value is time-variant) to reflect the changing effort in Little Moose Lake also did not produce increases in population abundance (Fig. 6).

Discussion

The population model that we developed is capable of producing the dynamic observed in response to the intensive removal of smallmouth bass from Little Moose Lake: an increase in the yearling and juvenile stages that leads to an increase in the total population abundance. The sensitivity analyses revealed that the value of α, the maximum per capita recruitment in the absence of density dependence, was a key factor in determining the response of the population to harvest. Changes in α produced the greatest changes in the population response to harvest, both in terms of whether or not an increase in population abundance was observed and also with regard to the magnitude of the increase. Although the model did not predict an increase in abundance using the parameters generated from the Lake Opeongo smallmouth bass population, small changes in maturation ($m_1 > 0.2$) or adult survivorship ($s_a \geq 0.7$) along with moderate increases in the maximum reproductive rate ($\alpha \geq 10$) would have resulted in increased equilibrium abundance with harvest. Although when parameter values were changed only slightly, the magnitude of the overcompensatory response (as predicted by the model) would be much smaller than what was observed in Little Moose Lake CPUE data.

The model demonstrated that commonly observed values of α reported in the fisheries literature (Myers 2001) could produce an increase in population abundance with harvest.

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Table 3. Maximum percent increase (as measured from the equilibrium population abundance in the absence of harvest) of total population size under harvest and the harvest level that produced the maximum for three harvest strategies: harvest of the total population, harvest of age-2 and older juveniles, and harvest of adults.

	Equal proportion harvest	Harvest of only age-2+ juveniles	Adult-only harvest
α			
10	0.5% at h = 3%	×	8.6% at h_a = 24%
15	7.3% at h = 11%	6.5% at h_j = 25%	27.2% at h_a = 42%
20	16.3% at h = 17%	16.5% at h_j = 37%	46.8% at h_a = 53%
25	25.7% at h = 21%	27.3% at h_j = 45%	66.1% at h_a = 60%
s_{j3}			
0.8	×	×	3.0% at h_a = 14%
0.9	×	×	8.7% at h_a = 25%
s_a			
0.7	×	×	1.8% at h_a = 8%
0.8	1.5% at h = 4%	×	8.5% at h_a = 15%
0.9	9.8% at h = 9%	1.9% at h_j = 31%	22.8% at h_a = 21%
m_1			
0.2	×	×	0.2% at h_a = 3%
0.3	×	×	1.1% at h_a = 8%
0.4	×	×	2.5% at h_a = 13%
0.5	×	×	4.1% at h_a = 18%
0.6	×	×	5.8% at h_a = 22%
0.7	0.3% at h = 3%	×	7.6% at h_a = 25%
0.8	0.8% at h = 5%	×	9.3% at h_a = 28%
0.9	1.4% at h = 7%	0.4% at h_j = 22%	11.0% at h_a = 31%
1.0	2.2% at h = 9%	1.7% at h_j = 35%	12.5% at h_a = 34%
m_2			
0.6	×	×	0.3% at h_a = 5%
0.7	×	×	0.9% at h_a = 8%
0.8	×	×	1.5% at h_a = 10%
0.9	×	×	2.2% at h_a = 12%
1	×	×	2.8% at h_a = 14%

Note: Example: when the parameter α was increased to 15 (while all other parameters were held constant at their estimated values), under the adult-only harvest strategy, the total population size was maximized at a 42% harvest of adults and the equilibrium population was 27.2% larger than in the absence of harvest. An × indicates that the population did not increase with harvest or that the percent increase was less that 0.1%.

In addition, Gross and Kapuscinski (1997) showed that greater than half of fall smallmouth bass recruits were produced by 5.4% of spawning males in a Lake Opeongo embayment, suggesting that very few individuals are capable of producing a large number of offspring. Yet, although α is an essential factor, this parameter alone cannot produce the dynamic of increased population abundance with harvest. The model demonstrated that the overcompensation in yearling and juvenile bass abundance was only observed when either high juvenile survivorship (s_j, s_{j2}, s_{j3} > 0.5) or high maturation (m_1 > 0.6) and adult survivorship (s_a > 0.7) rates occurred in conjunction with large per capita recruitment. Because high reproduction rates are generally associated with high mortality, this may, in part, be the reason why the overcompensatory response is not more widely observed in fish populations. Furthermore, the magnitude of the increase in population abundance with harvest was large (greater than 25% of the equilibrium abundance in the absence of harvest) only in cases where α was high ($\alpha \geq 15$ for the adult-only harvest strategy and $\alpha \geq 25$ for the equal proportion and age-2+ juvenile harvest strategies). It is possible that increases in population abundance with harvest may be more prevalent than previously expected but undetectable because the magnitude of the increases are very small or preharvest population information is unavailable.

When is a fish population likely to respond to harvest by increasing in abundance?

A population can only respond to harvest with an increase in abundance if at least one density-dependent relationship (i.e., in recruitment, survivorship, maturation, etc.) is overcompensatory. For example, if we had modeled our population with a typical Beverton–Holt recruitment function (Beverton and Holt 1957) in which recruitment saturates with increasing spawner abundance, projections would have shown that harvest caused a decline in abundance over the entire parameter space. An overcompensatory recruitment function with a steep slope near the origin (i.e., high value for α) is the most plausible hypothesis for an increase in population abundance in response to the intense harvest in Little Moose Lake.

Our model identified two scenarios under which an in-

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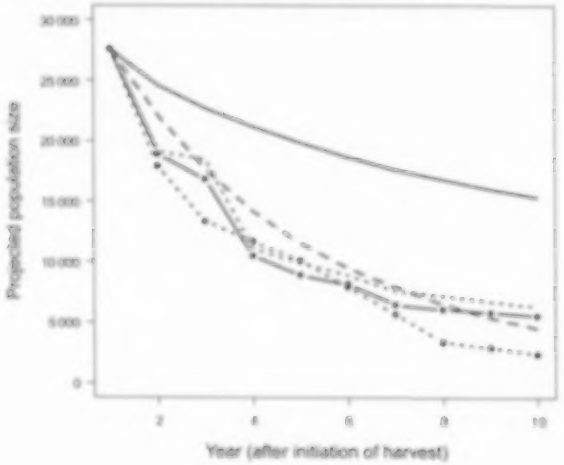
Fig. 6. Short-term dynamics for the population model under harvest using the Lake Opeongo parameter estimates. This graph shows the projected population size for 10 years under several harvest strategies: solid line, 15% harvest of all stages; long-dashed line, 10% harvest of age-1 individuals, 20% harvest of age-2 and older juveniles, and 50% harvest of adults; short-dashed line, equal harvest among all stages with proportion varying to reflect changing effort in the harvest intensity; solid line with points, varying harvest among the stages (to incorporate differences in potential catchability of individuals) and time varying to reflect changing effort; short-dashed line with points, randomly selected harvest for each stage (10%–40% for age-1 individuals, 10%–50% for age-2 and older juveniles, and 30%–70% for adults) in each year.



crease in population abundance could occur with harvest. The first (case 1) is characterized by midrange per capita recruitment in the absence of density dependence (approximately $5 \leq a \leq 10$), high maturation rate for young juveniles, *J*2 individuals ($m_1 > 0.6$), and high adult survivorship ($s_4 > 0.7$). The second (case 2) is a population with high per capita recruitment in the absence of density dependence (approximately for $a > 10$), high survivorship of immature fish ($s_1, s_{J2}, s_{J3} > 0.5$), and reasonably high annual maturation rates for older juveniles, *J*3 individuals ($m_2 > 0.2$).

We speculate that the first and second cases above are similar to the two population categories — maturation regulated and reproduction regulated — described by De Roos et al. (2007) in an analysis of overcompensation in a stage-structured biomass model. In their study, De Roos et al. (2007) suggested that when a population is maturation regulated, strong competition occurs among juveniles and therefore maturation is more resource-limited than reproduction. Within a reproduction-regulated population, competition is stronger among adults, and as a result, reproduction is more limited. Overcompensation in stage-specific biomass in response to harvest is possible in both scenarios (De Roos et al. 2007). We suggest that in our case 1 modeling scenario in which overcompensation occurs as a result of harvest, adult survivorship must be high, implying that competition may be more intense among juveniles than adults. The opposite is true in the case 2 scenario where juvenile survivorship must be high, suggesting that adult competition may be regulating the population at equilibrium.

In the case of Little Moose Lake, the smallmouth bass population may be regulated by competition among adults, based on slow growth rates and low levels of recruitment prior to the initiation of removal (Weidel et al. 2000). Analysis of diet data from the Little Moose Lake population showed that the ratio of adult to juvenile ingestion rates (defined as q by De Roos et al. (2007)) was less than one and has decreased since the onset of the removal (unpublished data), which further implies that adult competition may be an important factor driving the overcompensatory response of the yearling and juvenile stages. The model presented by De Roos et al. (2007) predicted that in a reproduction-regulated population, harvesting any or all stages of the population could result in an increase in juvenile biomass (as was observed empirically with respect to abundance). Additionally, the CPUE data from Little Moose Lake yields a much higher, if not unrealistic, value of a ($a \approx 84.3$). Available data from Little Moose Lake cannot be used to accurately generate stock–recruitment parameter values because of the short length of the time series and also because the catchability of smallmouth bass is likely length-dependent, which would bias estimates of a. Still, the very large estimated value of a from available data provides anecdotal evidence that the recruitment of smallmouth bass in Little Moose Lake may be large. The magnitude of the population increase in overall abundance shown in the CPUE data also suggests that the value of a may be high, given that sensitivity analyses revealed that the greatest increases in abundance occurred with the largest values of a. If adult competition in the Little Moose Lake bass population was released through harvest, per capita recruitment could be large, indicating a situation that is similar to the case 2 scenario (i.e., high per capita recruitment in the absence of density dependence, $a > 10$, and high survivorship of immature fish, $s_1, s_{J2}, s_{J3} > 0.5$).

Is there an optimal control harvest strategy?

The optimal harvest strategy is highly dependent on the control objective. If the goal of the harvest is to reduce the overall population abundance (regardless of demographic structure), then in situations in which the population's reproduction rate at low spawner abundance is large, harvest will not be effective until very high levels are achieved. In such cases, it may not be beneficial to remove any individuals unless it is possible to remove nearly all of the population. In other situations, the structure of the population may be of greater importance. For example, increased abundance of large smallmouth bass (>200 mm) in Little Moose Lake has altered food web linkages and has had a measured impact on the abundance of other littoral fish species (Lepak et al. 2006; Weidel et al. 2007). Therefore a key management goal is to minimize the impact of large bass (and reduce the total biomass of the bass) rather than simply reduce the overall population abundance. In such cases, a management tradeoff exists between a reduction in abundance of one life stage and a potential overcompensatory response in another. In this situation, continued regular harvesting may be necessary to maintain a reduction in adults.

If it remains impossible to eliminate all bass from the

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lake, the best Little Moose Lake smallmouth bass management strategy may be to reduce the proportion of adult fish (>200 mm) that are harvested. The sensitivity analyses showed that removal of adults caused the largest increase in yearling and juvenile abundances. Because we do not have annual population abundance estimates, it is difficult to determine exactly how many adults should be harvested to both achieve the management objectives described above and not produce an overcompensation of yearlings or juveniles. However, some reduction in the harvest of adults would likely mitigate the observed overcompensatory response without producing large increases in adult abundance, as well as reduce the effort necessary to maintain the positive effects of the removal.

Observations with Lake Opeongo parameter values

Implementing the model with parameter values generated from the Lake Opeongo smallmouth bass population did not produce an increase in total population abundance (or in any of the stages) under the harvesting scenarios that we evaluated. However, our results indicated that slight changes in individual parameter values can produce an increase in population abundance under three (age-2 and older juvenile only, adult only, and all stages in equal proportion) of the four harvest strategies that we examined. Several potential reasons could account for this apparent discrepancy. First, the 54 years of data from Lake Opeongo encompass a period in which smallmouth bass invaded and began to colonize the lake following their introduction in the 1920s (Shuter and Ridgway 2002). When creel surveys were initiated in 1937, smallmouth bass were still becoming established in this large lake. The stock–recruitment relationship could have been fundamentally different during the colonization period compared with the subsequent time period in which the population had become well established. This is evidenced by the value for α being higher ($\alpha = 9.3$) when we re-estimated the stock–recruit relationship excluding data from the first 20 years of data collection. Second, the parameter values are time-invariant in the model. We averaged annual survivorship and maturation to produce a constant value for each parameter, which is a very restrictive assumption. In natural systems, these rates vary annually and can be affected by a number of biotic and abiotic factors. For example, juvenile survivorship and growth of smallmouth bass is dependent on both population abundance and summer air temperatures (Shuter and Ridgway 2002); therefore the average values used in our model ignored interannual variation. In addition, the natural mortality parameter for adult survivorship included recreational fishing mortality. If the parameter s_a was re-estimated excluding the fishing mortality, adult survivorship would be higher. Increasing adult survivorship would result in an increase in abundance with harvest but that alone could not produce the magnitude of the increase that was observed in the smallmouth bass CPUE data from Little Moose Lake. Finally, it is possible that there are fundamental differences between the smallmouth bass populations in Lake Opeongo and Little Moose Lake. One indication that the stock–recruitment relationship may be different in the the two lakes is evident in the large difference in the values of α generated by the CPUE data from Little Moose Lake ($\alpha = 84.3$) and the data from Lake Opeongo ($\alpha = 5.5$). Although we cannot confidently assess the validity of the Little Moose Lake value (for reasons previously described), the very high value of α suggests that smallmouth bass recruitment in Little Moose Lake may be substantially different from that in Lake Opeongo.

It is possible that the CPUE data from Little Moose Lake do not accurately reflect the changes in bass abundance. Although we tried several methods to estimate yearly abundances, including depletion and cohort analyses, we were unable to develop robust population estimates because, in part, CPUE data and population abundances are not likely related by a simple factor (Schoenebeck and Hansen 2005). Although harvest effort has varied over the eight removal years, the methods for the removal have remained constant and the intensity of the removal has remained high. At the initiation of the harvest, 5014 smallmouth bass were removed in the spring of 2000. The cohort analysis estimated that there were approximately 7000 individuals present during the initial harvest. This suggests that nearly 72% of bass that were present were removed in the spring of 2000. As a result, we believe that the CPUE data can serve as a proxy for establishing that the population is responding to harvest by overcompensating.

Model extensions

The sensitivity analysis results in part reflect how density dependence was incorporated into the model. The decision to include density dependence in the stock–recruitment relationship (rather than in another stage or in multiple stages) was based on empirical studies of smallmouth bass life history and population dynamics that demonstrated the presence of density dependence in the recruitment process (DeAngelis et al. 1993; Dong and DeAngelis 1998; Shuter and Ridgway 2002). DeAngelis et al. (1991) used an individual-based model to show that the relationship between the number of smallmouth bass nests and the number of yearling recruits was overcompensatory, which they described as "not unlike a Ricker (1954) curve". DeAngelis et al. (1993) found a similar Ricker-like relationship between the number of fertilized eggs and the number of age-0 smallmouth bass that survived through the early part of the growing season. Dong and DeAngelis (1998) further showed that cannibalism and intense intraclass and interclass competition within a smallmouth bass population can lead to overcompensation in the recruitment of age-0 fish.

In addition, studies on juvenile and adult smallmouth bass suggested that there may be a density-dependent relationship between the number of mature fish and the number of fish that actually spawn (Raffetto et al. 1990; Ridgway et al. 1991; Shuter and Ridgway 2002). Ridgway et al. (2002) developed the juvenile transition hypothesis to describe their observation that as the population of mature males increases, the proportion that spawn declines (Ridgway et al. 1991; Ridgway et al. 2002; Shuter and Ridgway 2002). To incorporate this observation, we modified the equation for the yearling stage (eq. 1) to include a saturation function for the number of adults that spawn:

$$(7) \qquad Y_{t+1} = \frac{A_t(1-h_a)}{1+\delta A_t(1-h_a)}\alpha e^{-\frac{h_y+\delta h_y}{1+\delta A_t(1-h_a)}}$$

The parameter δ controls the negative effects of increasing

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the number of adults on the proportion that spawn. During the parameterization process, the best fit to the Lake Opeongo stock–recruit data occurred when $\delta = 0$, in which case the model simplifies to a standard Ricker recruitment function. Therefore, we did not use this modified stock–recruitment relationship for further analyses.

Observations of the smallmouth bass population in Little Moose Lake suggested that size at maturity has remained fairly constant but length at age has increased since the onset of the removal (unpublished data). This suggests that maturation rates may have increased over the time period of the removal, which begs the question as to whether this change would be enough to drive an overcompensatory response in the bass population. We looked at this scenario by modifying the maturation rate of age-3 individuals, the parameter m_3. Because this parameter was sensitive at low values of α (and α was estimated at approximately 5.5 through the parameterization process), we speculated that if the maturation rate of *J2* individuals was a density-dependent function of the number of adults in the population, then perhaps that parameter could be driving the observed increase in population abundance. The revised model included the time-variant maturation parameter:

$$m_{3,t+1} = m_{min} + \frac{m_{add}}{1 + \mu A_t} \quad (8)$$

where m_{min} is the maturation rate when the adult population is large (we set m_{min} to the estimated value for m_3 from the parameterization process), m_{add} is the maximum addition onto m_{min}, and μ regulates the effects of adult population density on m_{add}. Though we did not have data to parameterize m_{add} and μ, we were able to determine that even under a "favorable" parameterization (i.e., one in which $m_{3,t+1}$ reached high values with even modest reductions to A), the addition of a density-dependent time-variant maturation rate (when the value of $m_{min} = m_3$) was not enough by itself to lead to overcompensation with harvest. The major variation in the results with this revised model was that as harvest was increased, it became increasingly difficult to collapse the population.

We focused our analyses on equilibrium dynamics in response to harvest because we were interested in the long-term response of smallmouth bass to an intense harvest. However, further analyses could focus on the short-term dynamics of the Little Moose Lake study population and time-varying harvests to better understand the mechanisms responsible for population-level overcompensation in response to intense harvest. The presence of a "saw tooth" temporal pattern in young-of-the-year and yearling abundance is consistent with alternating strong year classes suppressing each subsequent year class. This could occur as a result of intense competition between the yearling and juvenile stages or through cannibalism, both of which have been previously used to justify use of the Ricker recruitment curve (Claessen et al. 2004). Our results support the use of the Ricker equation, but further analyses could explicitly incorporate these processes into the model.

Our model, together with empirical observations from the Little Moose Lake smallmouth bass population, demonstrates the potential for fish population abundance to increase in response to harvest. Although an increase in stage-specific or population-level abundance in response to harvest has been reported for a number of taxa (Slobodkin and Richman 1956; Buckley et al. 2001; Cameron and Benton 2004) and discussed theoretically (Dennis et al. 1997; Abrams and Matsuda 2005; De Roos et al. 2007), this type of response has not been previously documented empirically within a fisheries context. Studies of fish population responses to perturbations have shown that when overcompensation has occurred, it was in the form of individual growth or maturation rates (Weatherley and Gill 1981; Miglavs and Jobling 1989; DeAngelis et al. 1993). Our results show that one potential response of fish populations to harvest can be overcompensation in stage-specific abundances, which can subsequently lead to an overall increase in total population abundance. This is especially important to consider when the objective of harvest is control. In some instances, control through harvest may not be a feasible strategy. Alternatively, observed increases in population abundance may be a temporary result in situations in which complete mortality of all life stages cannot be quickly imposed; yet this response might ultimately disappear as control measures become more effective. Overall, our results show that the decision to reduce abundance through harvest must take into consideration the demographic characteristics and the density-dependent processes of the targeted population.

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The influence of food quality and availability on trophic position, carbon signature, and growth rate of an omnivorous crayfish

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Abstract: Fourteen sites in 13 streams were surveyed to address the importance of food quality and quantity on trophic position, carbon signature, and growth rate of noble crayfish (*Astacus astacus*) by combining the analyses of gut content, stable isotopes, and RNA–DNA ratio. The gut content analysis showed an omnivorous feeding habit, but total animal food sources contributed more to the nitrogen and carbon isotope signals of this species. The biomass of invertebrates (i.e., high quality food) influenced the trophic position, carbon signature, and growth rate. Our results show that crayfish in streams with a high biomass of invertebrates were at a higher trophic position and had a higher growth rate than crayfish in streams with a low biomass of invertebrates. A high biomass of relatively sedentary invertebrates indicates a habitat with high algae biomass, which is reflected in the more autochthonous carbon signature of crayfish. Further, noble crayfish in temperate streams seem to have dual functional roles by acting as predator and as shredder by processing large amounts of detritus.

Résumé : Nous avons inventorié 14 sites dans 13 cours d'eau pour déterminer l'importance de la qualité et la quantité de la nourriture sur la position trophique, la signature de carbone et le taux de croissance de l'écrevisse à pattes rouges (*Astacus astacus*) en combinant des analyses du contenu du tube digestif, des isotopes stables et des rapports ARN–ADN. L'analyse du contenu du tube digestif révèle des habitudes alimentaires d'omnivore, mais l'ensemble des sources alimentaires animales contribuent plus aux signaux isotopiques d'azote et de carbone chez cette espèce. La biomasse des invertébrés (c'est-à-dire de la nourriture de haute qualité) influence la position trophique, la signature de carbone et le taux de croissance. Nos résultats montrent que les écrevisses dans les cours d'eau à forte biomasse d'invertébrés occupent une position trophique supérieure et ont un taux de croissance plus élevé que les écrevisses dans les cours d'eau à faible biomasse d'invertébrés. Une importante biomasse d'invertébrés relativement sédentaires représente un habitat avec une forte biomasse d'algues, ce qui se reflète dans la signature de carbone plus autochtone des écrevisses. De plus, l'écrevisse à pattes rouges dans les cours d'eau tempérés semble jouer deux rôles fonctionnels, en agissant comme prédateur et aussi comme déchiqueteur responsable du traitement de grandes quantités de détritus.

[Traduit par la Rédaction]

Introduction

Omnivory, the consumption of resources from more than one trophic level, is common (Polis et al. 1989; Diehl 1993), and omnivores such as crayfish exist in most freshwater ecosystems. The diet of omnivores has been proposed to be influenced by the relative abundance and quality of food types at different trophic levels (Diehl 2003), but also by encounter rates and capture success (Rosenheim and Corbett 2003).

Omnivorous crayfish can dominate the benthic biomass (Momot et al. 1978; Rabeni et al. 1995), and as omnivores, they can affect several trophic levels both directly and indirectly (Nyström et al. 1996). Several studies indicate selective feeding by crayfish. Alcorlo et al. (2004) showed that the crayfish *Procambarus clarkii* consumed animal prey according to the latter's availability in the environment. Parkyn et al. (2001) showed a similar result in which the percentage of invertebrate groups consumed by the crayfish *Pharanephrops planifrons* largely reflected the invertebrate composition in the environment. However, sedentary taxa such as chironomids and gastropod snails constituted a higher proportion in the diet than available in the environment (Parkyn et al. 2001), indicating selective feeding on these groups by crayfish. Several other studies also show that large sedentary or less mobile species are commonly consumed by crayfish, whereas fast-swimming invertebrates are less abundant in crayfish guts (Abrahamsson 1966; Whitledge and Rabeni 1997; Nyström et al. 1999).

Most gut content analyses show that crayfish are omni-

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vores, consuming detritus, macrophytes, periphyton, invertebrates, fishes, and even other crayfish (Whitledge and Rabeni 1997; Parkyn et al. 2001; Stenroth and Nyström 2003). Gut content analysis gives information on recent crayfish feeding activity (Gherardi et al. 2004) but has several shortcomings. Different food items are digested at different rates (Momot 1995; Nyström 2002), and in addition, gut content analysis may not show long-term feeding habits (i.e., trophic position). To circumvent this problem, stable isotope analysis commonly has been used. Stable isotope ratios give information on assimilated food sources over long time periods; hence, they are used to identify important food sources for consumers including crayfish (Nyström 2002). A number of stable isotope analyses of crayfish have pointed to detritus as an important food source (Bunn and Boon 1993; France 1996; Evans-White et al. 2001). On the other hand, studies have also shown that invertebrates are the most important food source for crayfish (Parkyn et al. 2001; Hollows et al. 2002; Nyström et al. 2006). Lancaster and Waldron (2001) argue that crayfish, as omnivores, would encounter signatures from all food sources consumed and therefore it can be hard to distinguish the contribution of individual food sources to the isotopic signals in crayfish. However, it should be possible to distinguish between the contributions of different food sources to the isotopic signals in crayfish by combining stable isotope analysis and mixing models.

Further, increased primary productivity may increase the trophic position of crayfish by supporting higher densities of invertebrates (Stenroth et al. 2006). In streams, not only productivity but also characteristics such as canopy cover, water velocity, substrate, pH, and predation may affect the biomass and species composition of invertebrates (Orth and Maughan 1983; Biggs et al. 2000; Nyström et al. 2003). It is therefore possible that multiple abiotic and biotic factors influence resource supply for crayfish and hence their trophic position.

Crayfish feeding habits may influence not only trophic position, but also the individual growth rates of crayfish. Several abiotic factors, such as temperature, productivity, pH, calcium concentration, and habitat composition (Nyström 2002; Reynolds 2002), have been proposed to affect crayfish growth and individual size. Also, several biotic factors, such as food quality and availability (Momot 1995), predation risk (Nyström 2002), intraspecific interaction, and population density (Guan and Wiles 1999), would likely affect crayfish growth. D'Abramo and Robinson (1989) found that crayfish have a dietary protein requirement of at least 30%–35% for optimal growth. To meet their energetic demands, crayfish have to ingest animal food and do so by consuming the most profitable prey items available (Alcorlo et al. 2004). There are few studies on growth rates of freshwater species (Tardif et al. 2005) and in natural crayfish populations, mainly because of the lack of feasible methods to estimate growth in situ. Analysis of RNA–DNA ratios in muscle tissues can give information about growth rates and has been used successfully on many marine organisms, including lobsters (e.g., Parslow-Williams et al. 2001). Buckley (1984) consider the RNA–DNA ratio to be an instantaneous measurement of growth rate because it responds rather quickly to changes in feeding conditions and growth after 1–3 days. Therefore, it should be possible to address the relative growth rate of individual crayfish and compare individuals from different populations in freshwater ecosystems.

By combining gut contents, stable isotopes, and RNA–DNA ratios, it was possible to determine the feeding habits, trophic position, and carbon signature and its consequences for individual growth rates of omnivorous crayfish in different environments with different availability of high quality food (i.e., invertebrates). This study, conducted in multiple temperate streams, tests the following hypotheses. (*i*) Crayfish are omnivores according to gut content analysis, but animal food sources contribute more than plant material to the carbon and nitrogen isotope signals in crayfish. (*ii*) The availability of high quality food for crayfish is influenced by abiotic and biotic factors, and availability of high quality food affects the trophic position and carbon signature of crayfish. (*iii*) The growth rate of crayfish is influenced by the availability of high quality food. Therefore, crayfish occupying a habitat with a low biomass of invertebrates would have a lower growth rate (RNA–DNA ratios) than crayfish in habitats with high invertebrate biomass.

Materials and methods

We surveyed 13 streams in southern Sweden between August and September 2005. At one stream, two different sites were surveyed that differed substantially in stream characteristics and were more than 1 km apart. These sites were therefore considered as independent, which gave a total of 14 sites. Streams with established crayfish populations that differed in characteristics and location (forest, agricultural land, or pasture) were chosen. At each site, a study reach 8.3–55 m long, depending on width and availability, was investigated. The mean stream area investigated was 140 m^2 (range 60.3–346.8 m^2). Water temperature, oxygen levels, water velocity, canopy cover, and macrophyte cover were determined at each site. Water samples were frozen for later analysis of calcium, total nitrogen, total phosphorus, dissolved organic carbon (DOC), and pH.

To sample epilithon for biomass and stable isotope analysis, five cobbles were haphazardly collected within each study area and a 6.16 cm^2 circle of organic layer (i.e., epilithon, decomposed organic material, and microbes) was removed from the upper surface of each cobble with a wire brush, filtered onto a glass fibre filter (GF/C Whatman®), and frozen. Chlorophyll *a* was then extracted in ethanol (12 h in darkness at 20 °C) and estimated spectrophotometrically according to Jespersen and Christoffersen (1987). Samples of the organic layer for stable isotope analysis were prepared with a similar procedure, except for the chlorophyll *a* extraction.

Abundance and size distribution of noble crayfish (*Astacus astacus*) were estimated using traps. At each site, 30 Lini traps (crayfish ≥60 mm total length are caught in this type of trap) were baited with pieces of frozen cyprinid fish, following Swedish standard sampling methods for crayfish (Edsman and Söderbäck 1999). The number of crayfish caught per trap and night (catch-per-unit-effort, CPUE) was used as a relative measure of crayfish abundance. Traps that contained predatory fish or had been repositioned by the

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water current in a way that prevented crayfish from entering were excluded (Table 1). One hundred haphazardly chosen crayfish (or all when less was caught) were individually weighed to the nearest 0.1 g and measured to the nearest millimetre (total length, TL). From each population, 14 crayfish were collected (70–120 mm TL) and samples of abdominal white-muscle tissue were immediately frozen in liquid nitrogen for RNA–DNA analysis. The remaining parts were frozen (–20 °C) for analysis of gut content and stable isotopes (carbon and nitrogen).

The abundance and biomass of fishes were estimated by electrofishing (three depletion passes) a mean area of 140 m^2 (range 60.3–346.8 m^2). All fishes were identified to species, weighed to the nearest 0.1 g, and measured to the nearest millimetre (TL) prior to release. At the 12 sites where carnivorous fishes were caught, we collected 3–10 fishes considered to be predators on crayfish and other invertebrates (*Salmo trutta*, *Perca fluviatilis*, *Cottus gobio*, *Lota lota*, *Esox lucius*, and *Anguilla anguilla*) (Nyström 2002) for stable isotope analysis. In total, 65 individuals were collected and analysed.

To estimate the availability of high quality food (i.e., benthic invertebrate biomass), five surber samples (0.09 m^2, 250 mm mesh size) were taken at each site. Invertebrates were preserved in 95% ethanol and identified to the lowest possible taxonomic level (after Nilsson 1996, 1997) and counted at up to 10× magnification. To determine total invertebrate biomass, all individuals belonging to an invertebrate species were dried for 24 h at 60 °C and weighed to the nearest 0.1 mg and then combusted in a muffle furnace at 450 °C for 4 h and reweighed (ash-free dry weight, AFDW). Samples for stable isotope analysis were collected separately with a surber sampler or by searching cobbles and vegetation. Invertebrates were categorized into functional feeding groups (FFG; predatory invertebrates, grazers, shredders, and collectors) after Elliott et al. (1988), Nilsson (1996, 1997), Wallace et al. (2003), and Edington and Hildrew (2005) to decrease the number of food sources in our analyses (a priori aggregation of sources that have similar isotopic signatures and are logically related, e.g., Phillips et al. 2005). All invertebrates were frozen for later stable isotope analysis (3–20 animals from each FFG per stream).

Gut content and stable isotope analysis

A total of 90 crayfish guts, haphazardly chosen from the 14 sites (6 to 10 from each site), were examined under a binocular microscope (10× magnification). Organic matter was separated into detritus, wood, algae, and green plant fragments, and invertebrates were identified to generic level when possible, following Nilsson (1996, 1997), or classified as unidentified animal remains. The frequency of occurrence of food items in crayfish guts was calculated from the data.

In food web studies, the most commonly used elements for stable isotope analysis are carbon and nitrogen (Whitledge and Rabeni 1997). The carbon isotopic ratio ($^{13}C/^{12}C$) reflects assimilated food items and the isotopic enrichment from one trophic level to the next is often insignificant (Post 2002). The nitrogen isotopic ratio ($^{15}N/^{14}N$), on the other hand, typically increases on average 3.4‰ during each trophic transfer (Post 2002). For example, if predatory invertebrates are an important energy source for crayfish, their carbon isotopic ratios should be similar, whereas crayfish should have a nitrogen isotopic ratio about 3.4‰ above that of predatory invertebrates.

Stable isotope ratios of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) were obtained for crayfish, predatory fishes, shredding invertebrates (Capniidae and Leuctridae), grazing invertebrates (Baetidae and Gastropoda), collector invertebrates (Simuliidae and Hydropsychidae), predatory invertebrates (free-living Trichoptera, Hirudinea, Zygoptera, and Anisoptera), organic layer, detritus, and live plant material (filamentous algae and macrophytes). Samples for analysis consisted of untreated abdominal tissue of crayfish (as recommended by Stenroth et al. 2006), a tissue sample taken just behind the head of predatory fishes, abdominal tissue, or whole-body samples of invertebrates and a 0.5 cm × 0.5 cm sample of organic layer, detritus, and live plant material (approximately 0.2 mg of carbon). Samples were dried at 60 °C for at least 1 h and analysed with a PDZ Europa Ltd. (Sandbach, Cheshire, UK) 20–20 stable isotope analyser. The δ notation is the relative difference between samples and known standards:

$$\delta X = ((R_{sample}/R_{standard}) - 1) \times 1000$$

where X is either ^{15}N or ^{13}C and the corresponding ratio (R) is either $^{15}N/^{14}N$ or $^{13}C/^{12}C$. Atmospheric nitrogen is used as the standard ratio for nitrogen, and Vienna Pee Dee Belemnite is used as the standard ratio for carbon. Glycin was used as internal standard, and the internal precision for the standards was <0.3‰ for both C and N in each run. All isotope values are given in per mille (‰).

Growth rate

The RNA content of a cell is positively related to the amount of protein synthesis in the cell and thus to growth rate, whereas DNA content is constant (Clemmesen 1994), so the RNA–DNA ratio enables comparison of relative growth rates among populations. Analyses of RNA–DNA ratios were made according to the protocol for zooplankton of Vrede et al. (2002) with the following modifications: RNA-later was not used, RNA and DNA were extracted from approximately 1 mg of abdominal white-muscle tissue (Moss 1994, Parslow-Williams et al. 2001) in 1 mL of buffer from which 10 mL subsamples were taken and diluted 10-fold before being used in the analysis. The samples were then heated at 60 °C in a water bath to inactivate naturally present RNase (Weatherford et al. 1972; Pasloske and Wu 2004) for 10 min prior to 1 h of shaking. After the first reading (RNA and DNA), RNase was added to eliminate RNA and the samples were incubated in darkness for 20 min before the second reading (only DNA). RNA–DNA ratios of duplicate samples of homogenized tissue were determined with a Fluostar spectrometer. Ratios for 8 to 10 crayfish of the 14 collected (70–120 mm, TL) were determined for each site.

Statistical analysis

We tested if the biomasses of different invertebrate groups were related to any of the measured environmental factors in a canonical correspondence analysis (CCA) by multivariate constrained ordination (Leps and Smilauer 2003). Environmental factors with an absolute loading ex-

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Table 1. Descriptive physicochemical and biotic data for the 14 sites.

Site no.	Stream name	Study area (m^2)	Water temp. (°C)	pH	Ca ($mg \cdot L^{-1}$)	Chl *a* levels ($\mu g \cdot L^{-1}$)	Canopy cover (%)	Macrophyte cover (%)	Oxygen ($mg \cdot L^{-1}$)
1	Hänälven	75	17.7	6.9	6.6	1.27	75	2	9.1
2	Rommenäs	111	15.1	6.4	4.1	0.99	20	10	10.1
3	Hämnäs	123	15.9	6.4	4.0	2.17	2	30	9.5
4	Bärhus	144	18.6	7.2	6.4	0.20	85	0	8.9
5	Lillälven	347	17.4	6.9	3.8	0.92	3	45	9.4
6	Stömne I	178	17.4	7.0	5.8	1.35	90	0	9.2
7	Steneby	147	17.1	6.8	4.8	1.37	85	1	9.3
8	Stömne II	60	16.6	6.9	5.5	4.01	50	20	9.2
9	Töftedal	140	15.5	6.7	8.9	1.67	2	10	9.6
10	Sågän	220	17.6	6.7	8.8	1.97	90	0	8.2
11	Bodane	183	16.4	6.1	5.1	1.59	40	50	7.7
12	Ö. Derome	92	18.5	7.0	9.6	1.47	85	10	6.6
13	Hämnäs trib.	92	16.7	6.3	6.6	3.42	40	5	7.3
14	Faxeröd	48	16.7	6.9	10.9	0.20	98	2	8.8

Note: Chl *a*, chlorophyll *a*; DOC, dissolved organic carbon; temp., temperature; trib., tributary. Mean invertebrate biomass was calculated from the surber calculation, which are also shown in the table.

ceeding 0.5 were considered important. To ensure that the data on biomasses of invertebrate groups were normally distributed, they were $\ln(x + 1)$-transformed prior to analysis.

To examine how the different food sources contributed to the isotopic signatures of crayfish, a separate IsoSource mixing model was used for each element (Phillips and Gregg 2003). A mass balance tolerance of 0.05‰ and a source increment of 2% were used to avoid impractical amounts of computation when using seven sources (Phillips and Gregg 2003). No solutions could be computed for nitrogen at two sites and for carbon at one site. This was probably because there were additional unsampled food sources or differences in turnover rate or fractionation. Nitrogen isotopic ratios of crayfish were adjusted for trophic enrichment by subtracting 3.4‰ from each value prior to analysis (Post 2002). Correction for carbon fractionation (–0.39, as recommended by Post 2002) had no effect on the results, so no correction was used. We used one-way analysis of variance (ANOVA) to test if there was a difference in contribution among the different food sources to the carbon or nitrogen isotope signals in crayfish at each site at which a solution was generated. Significant differences in contribution among food sources were followed by Tukey's post hoc test. Further, the contribution of all animal food sources together against all plant food sources together was tested by the Wilcoxon test for both carbon and nitrogen isotope signals in crayfish.

Trophic position of noble crayfish was calculated using the following equation:

$$TP_{cf} = ((\delta^{15}N_{cf} - \delta^{15}N_{baseline})/3.4) + 2$$

where TP_{cf} is the trophic position of noble crayfish, $\delta^{15}N_{cf}$ is the isotopic ratio of crayfish, $\delta^{15}N_{baseline}$ is the isotopic ratio of primary consumers, one trophic level increment in $\delta^{15}N$ is 3.4, and the trophic position of the organism used to estimate the baseline (i.e., primary consumer) is 2. We used the nitrogen signals of collector invertebrates as the baseline as we did not have nitrogen signals of more long-lived primary consumers, such as filter-feeding mussels, recommended as baseline by other studies (e.g., Cabana and Rasmussen 1996; Vander Zanden and Rasmussen 1999; Post 2002). Collector invertebrates were chosen as baseline because they had the lowest $\delta^{15}N$ signals of the primary consumers and were present at all sites, as recommended by Anderson and Cabana (2007).

To test if total invertebrate biomass influenced the trophic position (TP_{cf}), carbon signature ($\delta^{13}C$), and (or) the growth rate (RNA–DNA ratio) of crayfish, linear regressions were performed. To examine whether any specific invertebrate group could explain these relations, we reduced the number of invertebrate groups using principal component analysis (PCA) with varimax rotation. Invertebrate biomasses were $\ln(x + 1)$-transformed prior to analysis to ensure that they were normally distributed. Principal components (PC) with eigenvalues greater than one were retained, and variables with an absolute loading exceeding 0.5 were considered important (Quinn and Keough 2002). Stepwise linear regression was then used to test if any of the PC axes were related to the trophic position (TP_{cf}), carbon signature ($\delta^{13}C$), or growth rate (RNA–DNA ratios) of crayfish.

All statistical analyses were conducted in SPSS version 11.0 for Windows, except for the isotope-mixing model, which was conducted in IsoSource (www.epa.gov/web/pages/models.htm), and the canonical correspondence analysis (CCA), which was conducted in Canoco 4.0 for Windows.

Results

A total of 1977 noble crayfish was caught at the 14 sites, with the number per site ranging between 27 and 457. Crayfish density (CPUE) ranged from 0.9 to 15.2 individuals caught per trap and night. Predatory fish biomass ranged from 0 to 10.84 $g \cdot m^{-2}$, and invertebrate biomass ranged from 0.134 to 1.353 $g \cdot m^{-2}$. Abiotic factors such as canopy cover (range from 2% to 98%) and macrophyte cover (range from 0% to 50%) also differed greatly among sites. The concentration of total nitrogen had a mean of 0.426 $mg \cdot L^{-1}$, and the concentration of total phosphorus had a mean of 0.013 $mg \cdot L^{-1}$ (Table 1). Water temperature was above 15 °C, oxygen concentration was above 6 $mg \cdot L^{-1}$, and pH

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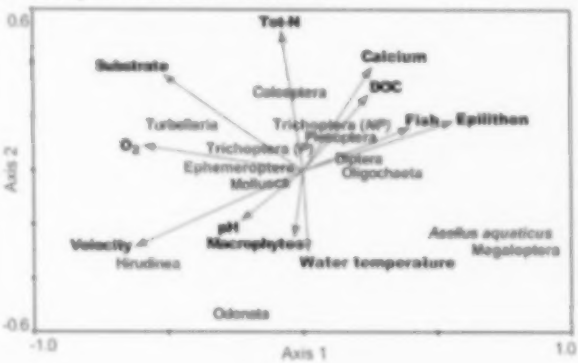
Total N ($mg \cdot L^{-1}$)	Total P ($mg \cdot L^{-1}$)	DOC ($mg \cdot L^{-1}$)	Velocity ($m \cdot s^{-1}$)	Total no. of crayfish	No. of traps	CPUE	Mean predatory fish biomass ($g \cdot m^{-2}$)	Mean invertebrate biomass ($g \cdot m^{-2}$)
0.33	0.005	8.45	0.08	231	30	7.7	0	0.325
0.51	0.006	7.98	0.68	105	28	3.8	0.68	0.409
0.40	0.015	10.10	0.16	261	29	9.0	2.85	0.073
0.33	0.005	8.41	0.35	69	30	2.3	1.16	0.289
0.29	0.005	5.94	0.92	155	29	5.3	0.15	0.217
0.30	0.005	5.59	0.09	102	29	3.5	4.13	0.111
0.28	0.006	8.38	0.18	79	30	2.6	0.09	0.060
0.31	0.007	6.07	0.21	65	28	2.3	8.84	0.162
0.67	0.014	17.70	0.17	27	29	0.9	10.84	0.105
—	0.050	8.71	0.40	147	29	5.1	3.26	0.610
0.67	0.026	21.90	0.07	457	30	15.2	1.47	0.063
0.35	0.005	5.40	0.13	107	25	4.3	10.48	0.175
0.49	0.027	25.70	0.20	128	20	6.4	0	0.165
0.61	0.005	15.50	0.22	44	30	1.5	7.33	0.288

samples. The crayfish abundance (catch-per-unit-effort, CPUE) is calculated from the total number of crayfish and the number of traps used in the

was above 6 at all sites (Table 1), which indicates good conditions for noble crayfish.

The CCA (Fig. 1) showed that the biomasses of invertebrate species were influenced by several environmental factors. The first axis explained 33.5% of the variation in invertebrate biomasses among sites and was correlated with oxygen (negatively), current velocity (negatively), substrate size (negatively), and epilithon biomass (chlorophyll *a*) (positively). The second axis explained 20.9% of the variation and was correlated mostly with total nitrogen (negatively). The biomasses of the different invertebrate groups were influenced by different environmental factors. For example, Diptera and Oligochaeta were mostly influenced by epilithon biomass (chlorophyll *a*) and the biomass of predatory fish (Fig. 1).

Fig. 1. Correlation plot of invertebrate groups to axes 1 and 2 from the canonical correspondence analysis (CCA), with the environmental factors that explained most of the variation. Trichoptera are divided into predatory (P) and nonpredatory (NP) taxa. DOC, dissolved organic carbon; Tot-N, total N.



Gut content and stable isotopes

The gut content analysis showed a varied diet in noble crayfish (Fig. 2). At all sites, 83% to 100% of the guts examined contained fish remains from the bait. Remains of plant food items and animal food items (fish remains excluded) were present in 94% and 52%, respectively, of the guts examined (empty guts excluded). The IsoSource mixing models indicate that noble crayfish derive their nitrogen mostly from invertebrates and their carbon from detritus, shredders, and predatory invertebrates (Figs. 3*a*, 3*b*). For the carbon isotope signals in crayfish, the ANOVA showed a significant difference in contribution between the different food sources ($F = 22.47$, df = 6, $p \leq 0.001$). Tukey's post hoc test showed that detritus and shredders contributed the most, followed by predatory invertebrates, whereas remaining sources did not contribute significantly to the carbon isotope signal of crayfish (Fig. 3*a*). The ANOVA showed no significant difference in percent contribution to the nitrogen isotope signal (adjusted for trophic transfer: $\delta^{15}N$ crayfish – 3.4‰) of crayfish between the different food sources ($F = 0.796$, df = 6, $p = 0.576$) (Fig. 3*b*). However, if all food sources of animal origin were pooled and all plant sources pooled (Figs. 3*a* and 3*b*), a trend could be seen that animal food sources contributed more to both carbon and nitrogen isotope signals in crayfish, even though this was not statistically significant (Wilcoxon test: for carbon, $Z = -0.91$, $p = 0.362$; for nitrogen, $Z = -1.67$, $p = 0.096$).

The dual-isotope plot on median values from all organism groups from the 14 sites show that noble crayfish are at the same trophic level as predatory fishes (Fig. 4). All invertebrate groups and filamentous algae are clustered together approximately one trophic level below crayfish and predatory fish, whereas the organic layers from cobbles and macrophytes are somewhat below and detritus is at the base of the food chain (Fig. 4). The carbon signal of crayfish in the dual-isotope plot looks like it is derived from the organic layer, shredders, and collectors, which is not in agreement with the results from the IsoSource mixing model. However, the organic layer consists of more than pure epilithon (i.e., chlorophyll *a*) and there was a significant correlation (linear regression, $F = 2.99$, df = 13, $p = 0.013$) between the measured chlorophyll *a* levels and the $\delta^{13}C$ of crayfish if the $\delta^{13}C$

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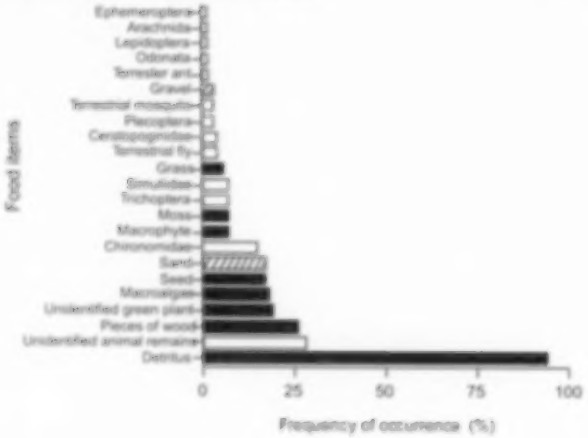
Fig. 2. Frequency of occurrence of different food items in 90 crayfish guts. Fish items were excluded as they were likely to originate from the bait. Solid bars, plant material; hatched bars, inorganic material; open bars, animal material.



of detritus was subtracted. This indicates that the carbon isotope signal of crayfish comes from detritus either directly or indirectly through the consumption of predatory invertebrates, shredders, and collectors.

Trophic position and carbon signature

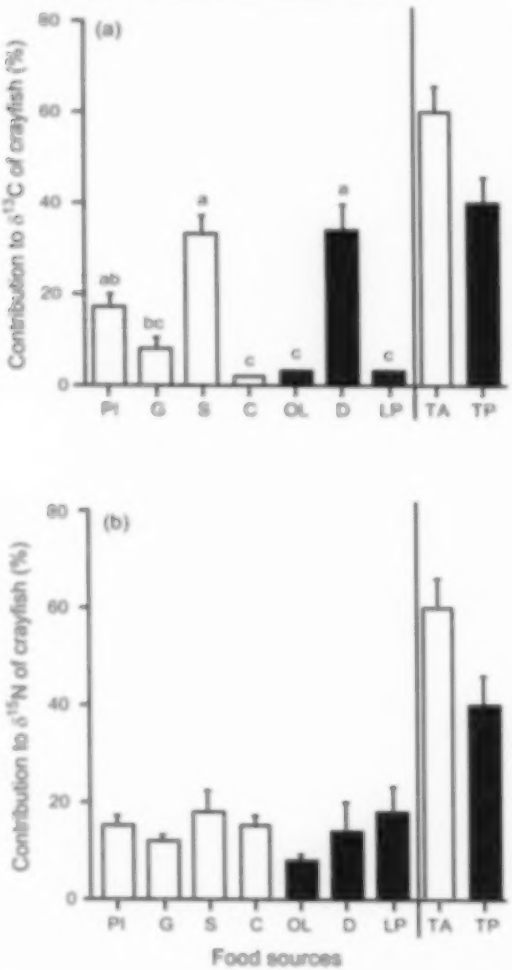
The mean $\delta^{15}N$ for all crayfish was 7.6‰ (range 5.8 to 9.3), and for $\delta^{13}C$, it was –27.5‰ (range –26.1 to –30). The standard deviation within crayfish populations for both nitrogen and carbon isotope values from the 14 sites varied from 0.24‰ to 0.94‰, with a mean of 0.59‰.

The trophic position (TP_{cf}) was positively related to invertebrate biomass (linear regression, $F = 8.11$, df = 13, $p = 0.015$). Crayfish at sites with a high biomass of invertebrates were at a higher trophic position than crayfish at sites with a low invertebrate biomass (Fig. 5*a*). The carbon signature ($\delta^{13}C$) of crayfish was not related to the biomass of invertebrates (linear regressions, $F = 0.003$, df = 13, $p = 0.96$) (Fig. 5*b*). However, the PCA derived five axes with eigenvalues greater than one (Table 2), and two axes were significantly related to the trophic position and carbon signature of crayfish. The first axis (PC1) represents biomasses of invertebrate groups that are commonly found in the crayfish diet (e.g., Ephemeroptera, Trichoptera, Plecoptera, and Mollusca), and the second axis (PC2) represents biomasses of groups that are relatively sedentary (e.g., Turbellaria, Oligochaeta, Coleoptera larvae, and Diptera) (Table 2). Stepwise linear regressions show that PC1 ($F = 5.03$, df = 13, $p = 0.045$) had an influence on the trophic position (TP_{cf}) of crayfish and PC2 had an influence on the carbon signature ($\delta^{13}C$) of crayfish ($F = 5.9$, df = 13, $p = 0.032$) (Table 2).

Growth rate

The growth rate (RNA–DNA ratio) of crayfish was positively related to the biomass of invertebrates, although not statistically significant (linear regression, $F = 3.958$, df = 13, $p = 0.07$). If, however, the water temperature was controlled for, there was a positive relation between RNA–DNA ratios of crayfish and the total invertebrate biomass (multiple linear regression, $F = 8.18$, df = 13, $p = 0.014$; Fig. 6*a*). There was also a positive relation between the trophic position (TP_{cf}) of crayfish and the RNA–DNA ratios of crayfish (multiple linear regression, $F = 5.625$, df = 13, $p = 0.035$) when the water temperature was controlled for. Crayfish at a higher trophic position had higher growth rates than those at a lower trophic position (Fig. 6*b*).

Fig. 3. The mean percentage contribution of the different food sources to (*a*) $\delta^{13}C$ and (*b*) adjusted $\delta^{15}N$ signals of noble crayfish. Different letters represent a significant difference at the 0.05 level (Tukey's post hoc test). Also the contributions of total animal food sources (open bars) and total plant food sources (solid bars) are shown to the right of the line. PI, predatory invertebrates; G, grazers; S, shredders; C, collectors; OL, organic layer; D, detritus; LP, live plant material; TA, total animal food sources; TP, total plant food sources. Error bars denote +1 standard error.



Discussion

Animal food sources together with detritus were the most important contributors to the nitrogen and carbon signals of

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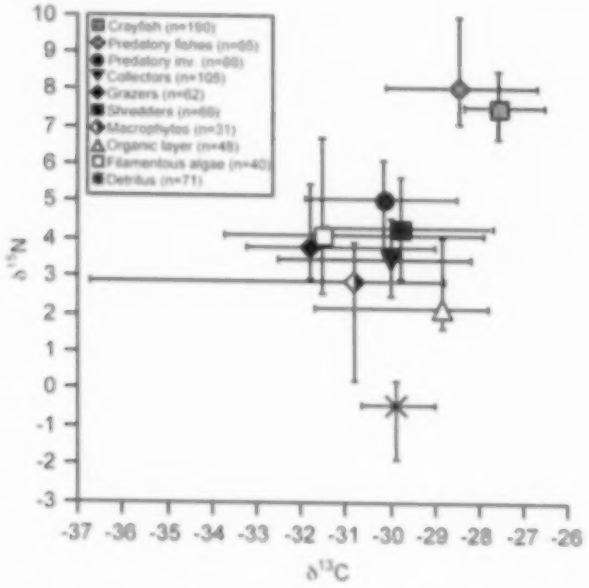
Fig. 4. Dual-isotope plot on median $\delta^{15}N$ and $\delta^{13}C$ from all sites combined showing signals in predatory fishes, noble crayfish, and potential food sources for crayfish. Live plants are divided into filamentous algae and macrophytes, as they differed substantially in carbon and nitrogen signals. Error bars denote the 25% and 75% quartiles.

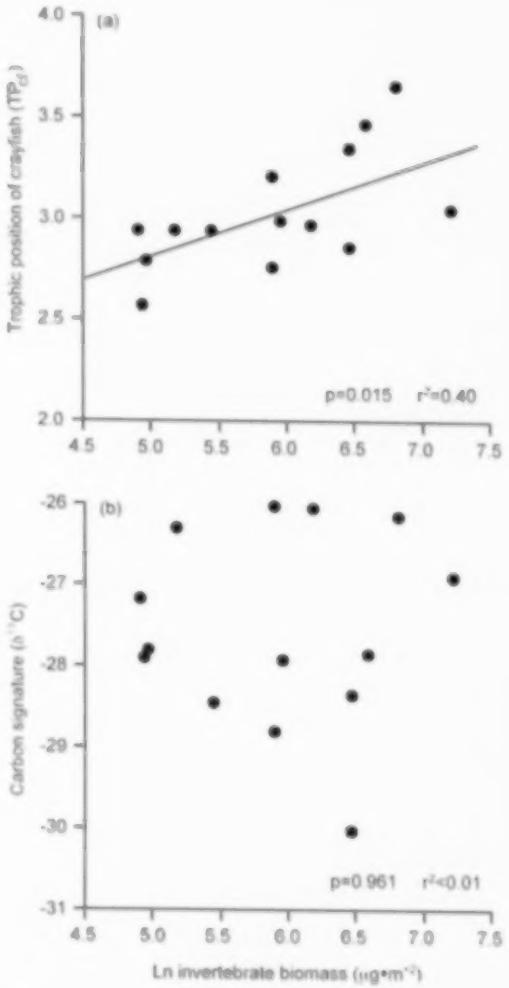


noble crayfish, even though detritus was found in 94% and animal food sources only in 50% of the crayfish guts examined. Crayfish held a position as top invertebrate predator in our study streams, and the availability of high quality food (i.e., biomass of invertebrates) explained both the trophic position and growth rate of noble crayfish. The biomass of the most commonly found invertebrate groups in crayfish guts influenced the trophic position of noble crayfish, whereas the biomass of relatively sedentary invertebrate groups gave an indication of the carbon origin of the system, which was shown in the carbon signature of noble crayfish. Overall, our results suggest that local variations in abiotic and biotic factors influence the biomass and species composition of invertebrates in temperate streams, which in turn affects the feeding of omnivorous crayfish and their subsequent trophic position and growth rate.

Gut content and stable isotopes

Noble crayfish in our study streams function as omnivores by consuming large amounts of detritus and invertebrates. This is consistent with several other studies of gut contents in crayfish (*Astacus astacus* (Abrahamsson 1966; Hessen and Skurdal 1986); *Pacifastacus leniusculus* (Stenroth and Nyström 2003); *Paranephrops zealandicus* (Hollows et al. 2002); *Paranephrops planifrons* (Parkyn et al. 2001; Olsson et al. 2006)), indicating an omnivorous feeding behaviour of crayfish in general. Several studies, including ours, show high amounts of detritus in crayfish guts, which might be due to the small energetic costs of consuming detritus and the microbial flora colonizing leaf litter (Momot et al. 1978; Parkyn et al. 2001; Roth et al. 2006). Parkyn et al. (2001)

Fig. 5. Linear regression between (*a*) trophic position (TP_{cf}) of crayfish and the biomass of invertebrates (ln-transformed) and (*b*) carbon signature of crayfish ($\delta^{13}C$) and the biomass of invertebrates.



and Roth et al. (2006) further argue that by consuming leaf litter, crayfish may gain enough energy for respiration and maintenance. However, more than 50% of the guts examined contained both animal and plant items and the models by Diehl (2003) predict that growth rate in omnivores is higher when feeding on both plant and animal food types compared with when feeding on either plant or animal items alone. It has been suggested that by consuming plant material, crayfish get essential minerals and nutrients such as carotenoids that are needed for pigmentation and thus necessary for optimal growth (Goddard 1988; Nyström 2002).

However, despite their omnivorous feeding habit, crayfish seem to occupy the trophic position of top invertebrate predator. The isotope data showed that noble crayfish are at the same trophic level as predatory fish, and animal material contributed more to the nitrogen isotope signals of noble

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Table 2. Component loadings for biomass of macroinvertebrate groups and percentage of variance explained by the four PC axes with eigenvalues greater than 1.

	PC1	PC2	PC3	PC4
Eigenvalue	4.8	2.3	1.5	1.4
% variance explained	37.2	18.0	11.6	10.7
Turbellaria	**0.658**	**0.594**	0.259	–0.278
Megaloptera	-9.1×10^{-2}	-8.8×10^{-2}	–0.240	**0.857**
Oligochaeta	1.3×10^{-3}	**0.885**	9.2×10^{-3}	0.178
Asellus aquaticus	–0.113	0.382	8.4×10^{-3}	**0.637**
Odonata	-2×10^{-2}	8.7×10^{-2}	**0.909**	6.7×10^{-3}
Ephemeroptera	**0.746**	4.13×10^{-2}	0.357	4.9×10^{-3}
Predatory Trichoptera	**0.871**	0.130	6.6×10^{-2}	–0.158
Nonpredatory Trichoptera	**0.796**	–0.139	2.8×10^{-2}	–0.130
Coleoptera	4.18×10^{-2}	**0.726**	–0.134	**–0.552**
Plecoptera	**0.811**	0.231	-2.5×10^{-2}	1.3×10^{-2}
Diptera	0.412	**0.726**	9.4×10^{-2}	0.217
Mollusca	**0.601**	0.238	**0.612**	-7.4×10^{-3}
Hirudinea	0.237	–0.215	**0.780**	–0.370
PC no. to carbon signature ($\delta^{13}C$), p =	0.933	**0.032**	0.129	0.871
PC no. to trophic position ($TP_{(f)}$), p =	**0.045**	0.083	0.194	0.316
PC no. to growth rate (RNA:DNA), p =	0.167	0.212	0.195	0.103

Note: Loadings given in bold type are considered to be important (absolute loading > 0.5); p values refer to stepwise linear regressions of carbon signature ($\delta^{13}C$), trophic position ($TP_{(f)}$), and RNA–DNA ratios of crayfish against the scores of the four axes.

crayfish than plant material. This is consistent with the findings by Parkyn et al. (2001), in which the dominant food items consumed by crayfish were invertebrates and detritus but the isotope data showed that crayfish (*Pharuneprops planifrons*) obtained most of their energy from invertebrates. Predatory invertebrates, shredders, and detritus contributed more than other food sources to the carbon isotope signal of crayfish according to the IsoSource mixing model, but from the dual-isotope plot, it looked like noble crayfish derived their carbon isotope signal from organic layer, shredders, and collectors. However, there was a positive relationship between the carbon isotope signals of crayfish, when the carbon signals of detritus were subtracted, and the chlorophyll *a* levels at our sites. This might have confounded the position of noble crayfish in the dual-isotope plot. The carbon signal of noble crayfish is likely to be derived mostly from detritus, either directly or indirectly by consumption of predatory invertebrates and shredders that were approximately one trophic level below noble crayfish and one trophic level above detritus in the dual-isotope plot. Parkyn et al. (2001) showed a similar result from pasture streams, where crayfish had a carbon isotope signal between epilithon and filamentous green algae through the consumption of mayflies and snails.

Trophic position and carbon signature

The biomass of invertebrates, which indicates prey availability, affected the trophic position of crayfish. The biomasses of certain invertebrate groups also correlated with the carbon signature of noble crayfish in our study streams.

A high trophic position of crayfish seemed to be related to high biomasses of Ephemeroptera, Turbellaria, Trichoptera, Plecoptera, and Mollusca. Moreover, these were the most commonly found invertebrate groups in the guts of noble crayfish. Many species among these groups are large and (or) slow-moving, which probably makes them easy for crayfish to catch in comparison with smaller and faster-moving invertebrates (Nyström et al. 1999; Stenroth and Nyström 2003). The carbon isotope signals in noble crayfish seemed to be related to the biomass of Turbellaria, Oligochaeta, Coleoptera, and Diptera. Crayfish had lighter carbon isotope signals in streams that had a high biomass of these four groups. Most species in these four invertebrate groups are considered to be relatively sedentary (Mellanby 1948) and will reflect the carbon signals of basal resources in streams. If the energy base is from allochthonous or autochthonous sources, this will be reflected in the carbon signal of sedentary species and transferred up the food chain. Parkyn et al. (2001) showed that the energy base for crayfish in forested streams originated from allochthonous sources, whereas the energy base for crayfish in pasture streams originated from both allochthonous and autochthonous sources. This is in agreement with our study in which the streams differed in canopy cover from 2% to 98% and represented both forested and pasture streams. Further, the biomass of these sedentary species is generally higher in more open habitats with higher algal standing crop (Sabater et al. 1997), and epilithon biomass (chlorophyll *a*) was the main environmental variable that influenced the biomass of Oligochaeta and Diptera in our study.

Selectivity for food sources in omnivorous crayfish has been reported in several other experimental studies (e.g., Hanson et al. 1990; Nyström et al. 1999), as well as under natural conditions (Lodge and Lorman 1987). Noble crayfish seem to be selective feeders as well, as specific invertebrate groups were responsible for the patterns found and the availability of high quality food determines the rate of selectivity

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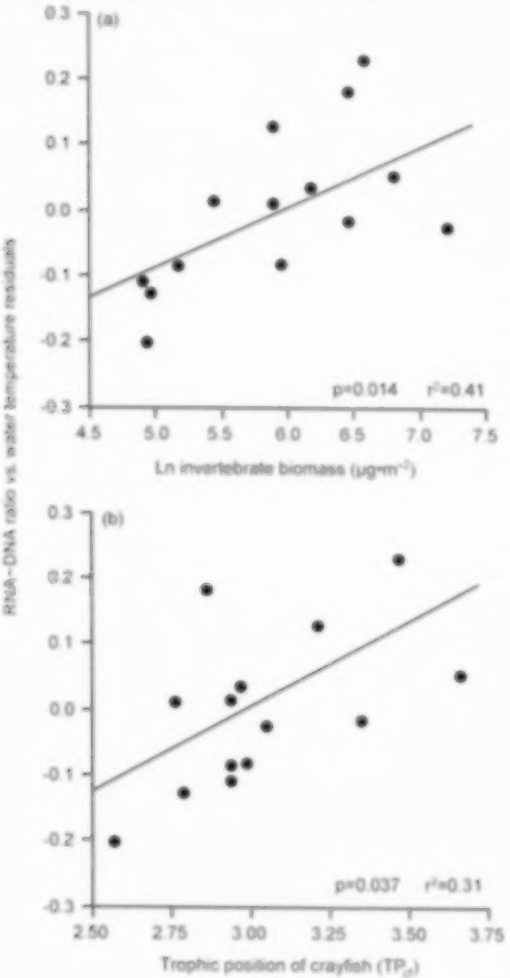
Fig. 6. Multiple linear regression between the growth rates (RNA–DNA ratio) of crayfish and (*a*) the biomass of invertebrates (ln-transformed) and (*b*) the trophic position (TP_{cf}) of crayfish. In both *a* and *b*, the water temperature was controlled for.



shown by crayfish. In a study by Correia (2002), crayfish (*Procambarus clarkii*) adjusted their trophic behaviour to the availability of invertebrates in the environment. Several other studies have also addressed the importance of animal food sources for crayfish and argued that the availability of specific invertebrates determines the consumption of high quality food (Momot 1995; Nyström 2002; Alcorlo et al. 2004). Our results show that crayfish in natural streams have a trophic position that reflects the availability of high quality food in the environment and that the carbon signature of crayfish reflects the carbon origin at the base of the system.

The use of stable isotopes and mixing models for estimating trophic position requires rather precise estimates of isotopic signatures in the baseline organisms or end members (Syväranta et al. 2006). Several studies have shown high spatial and temporal variability of isotope ratios in primary consumers (Vander Zanden and Rasmussen 1999; Anderson and Cabana 2007). Post (2002) states that obtaining an appropriate baseline is one of the most difficult tasks when using stable isotope techniques in food web analyses. Moreover, several primary consumer taxa are not strictly collectors, shredders, grazers, or predators but have a slightly omnivorous diet (Anderson and Cabana 2007), which increases the problem with choosing a proper baseline. Also, species can be strictly detritus feeders in one stream and more omnivorous in another, so caution should always be taken when interpreting results from stable isotope data in food web analysis. However, the use of stable isotope analysis and mixing models can give us a better understanding of the different roles of omnivores, such as crayfish, in food webs and their role for the flow of energy in stream ecosystems (Anderson and Cabana 2007).

Growth rate

Water temperature has been recognised as one of the most important factors affecting growth in crayfish (Lodge and Hill 1994; Reynolds 2002), but other variables such as food quality and availability may affect the growth rate if temperature is suitable for growth (Tardif et al. 2005). Our study shows that the biomass of invertebrates had an effect on the growth rates of crayfish when temperature effects were accounted for. Crayfish in streams with a high biomass of invertebrates had higher RNA–DNA ratios than those in streams with low biomass of invertebrates. However, there was no particular invertebrate group that was responsible for this pattern, in contrast to the trophic position and carbon signature of crayfish. It was rather the availability of high quality food per se that affected the growth rates of crayfish. Tardif et al. (2005) argue that higher growth in juvenile perch on the south shore of their study lake in summer may have been coupled with a larger quantity of benthos at the south shore compared with the other study areas. Several experimental studies have shown that crayfish growth rate is affected by food availability (Hill et al. 1993; Edsman et al. 1994; Paglianti and Gherardi 2004), including studies of noble crayfish (Nyström and Granéli 1996). Our results show that growth rate in this species is affected by food availability also in natural streams. Furthermore, there was a positive correlation between trophic position and growth rate. Crayfish at a higher trophic position had higher RNA–DNA ratios than those at a lower trophic position. Thus the availability of high quality food had a combined effect on the trophic position and growth rate of noble crayfish in our study streams. Crayfish at a higher trophic position consume more animal material than those at a lower trophic position and therefore will have higher growth rates.

Noble crayfish in this study had low RNA–DNA values compared with those of lobsters (Parslow-Williams et al. 2001). Several other studies record similar ratios to ours in fish larvae (Clemmesen 1996), barnacles (Desai and Anil 2002), and gastropods (Okumura et al. 2002). We therefore assume that the RNA–DNA ratios in noble crayfish in this study are what can be expected in natural stream populations of adult crayfish. It has been suggested that moult stages and stress when handling the organism could affect the RNA concentration and thus confound the results of RNA–DNA ratios. Moss (1994) showed that different moult

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stages did not affect the RNA–DNA ratios in juvenile shrimp (*Penaeus vannamei*), and Clemmesen (1996) argued that short time stressors related to catching procedure and handling does not affect RNA–DNA ratio. The RNA–DNA ratio is a quite conservative measurement and will give a good picture of the factors influencing an organism's growth and survival in a period for about a week prior to sampling (Clemmesen 1996). Further, an adult noble crayfish moults once or twice a year and will probably have a low growth rate compared with those of juveniles and species with a shorter life cycle. Moreover, the variation in growth rate within a population was very low, which indicates similar uptake of nutrients between individual crayfish. This is also consistent with the low variation in trophic position between individual crayfish from the same population, which indicates a similar diet in crayfish living in the same environment.

Overall, in this study, we have shown that noble crayfish derive most of their energy from animal food sources and occupy the trophic position of a top predator. The availability of invertebrates varies between streams because of differences in abiotic and biotic factors, and the biomass of invertebrates influences feeding, trophic position, and growth rate in noble crayfish. However, the availability of preferred invertebrate groups seems to partly explain some of the observed variation in trophic position of crayfish between streams. This could be because a stream supporting a high biomass of large, slow, and (or) sedentary invertebrates can enable a higher intake of animal resources for crayfish and thus place them at a higher trophic position. A higher trophic position (i.e., higher consumption of animal material) will also increase the growth rate of crayfish. Furthermore, a high biomass of sedentary invertebrates, such as Oligochaeta and Diptera, indicates a habitat with high algal biomass, which can be seen in the carbon signature of crayfish. In a habitat with a high biomass of these sedentary taxa, the carbon signature of crayfish is a mixture of both autochthonous and allochthonous sources compared with less productive habitats in which the carbon isotope signals of crayfish are solely from allochthonous sources.

By combining gut contents, stable isotopes, and RNA–DNA ratios, it is possible to address the importance of different food sources for the trophic position, carbon signature, and growth rate of omnivorous crayfish in different ecosystems.

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PERSPECTIVE / PERSPECTIVE

It's time to sharpen our definition of sustainable fisheries management

Peter A. Shelton and Alan F. Sinclair

Abstract: We review and evaluate the sustainability paradigm as it applies to wild capture fisheries in the context of a recently developed harvest strategy framework that, if implemented, will meet Canada's national and international obligations with regard to sustainable fisheries. This framework is based on an operationally explicit definition of sustainability that includes a commitment to managing for maximum sustainable yield. Although Canadian policy strongly supports sustainable fisheries management in principle, usage of the term has been vague and implementation of sustainabl; fisheries management strategies has lagged. Fisheries managed under the recently developed framework would be better able to meet new ecocertification and ecolabelling standards. An emerging governance structure discussed herein with respect to fisheries management is conducive to implementing sustainable management practices that meet long-term public good objectives.

Résumé : Nous passons en revue et évaluons le paradigme de la durabilité tel qu'il s'applique aux pêches basées sur la capture de stocks sauvages dans le contexte d'un cadre stratégique de récolte élaboré récemment qui, s'il est mis en oeuvre, permettra au Canada de réaliser ses obligations nationales et internationales relatives à la pêche durable. Ce cadre se base sur une définition explicite au niveau opérationnel de la durabilité qui inclut un engagement à faire la gestion de manière à atteindre un rendement durable maximal. Alors que la politique canadienne appuie fortement en principe la gestion durable des pêches, l'usage du terme est demeuré vague et la mise en oeuvre de stratégies de gestion de pêche durable a tardé. Les pêches gérées dans le cadre élaboré récemment devraient être plus à même de répondre aux nouvelles normes d'accréditation et d'étiquetage écologiques. La structure de gouvernance en émergence dont on discute ici pour la gestion des pêches devrait entraîner la mise en place de pratiques durables qui satisferont aux objectifs à longue échéance du bien commun.

[Traduit par la Rédaction]

Introduction

Canadian fisheries management policy has evolved over the past decade in response to increased national and international desire for more effective resource management. The collapse of Atlantic cod (*Gadus morhua*) in the early 1990s was preceded by a departure from a fixed exploitation rate strategy termed $F_{0.1}$ (Shelton 2007). The debate over an alternative strategy has been ongoing ever since. This has resulted in a surfeit of policy and a paucity of action. "Sustainable development" is generally accepted as the overarching objective for the management of naturally renewable resources such as fisheries, and Canada has made a clear commitment to this objective in numerous policy documents. We review and evaluate the sustainability paradigm as it applies to wild capture fisheries and describe a recently developed Canadian harvest strategy that is compliant with the precautionary approach (PA). We suggest that if made operationally explicit and widely implemented, this strategy would meet Canada's national and international obligations with regard to sustainable fisheries and precaution.

In addition to these scientific developments, there has been an important policy change on governance structures for fisheries management in Canada, away from the top-down model of the 1980s towards a more open and transparent process involving a broader representation of stakeholders. Institutional changes within Fisheries and Oceans Canada are underway to place greater emphasis on rights of First Nations and reflect the broader interests of society through increased participation in the decision-making process. A recent Federal Court of Appeal ruling in Canada (Larocque v. Canada (Minister of Fisheries and Oceans Canada); Federal Court of Appeal Canada 2006) re-established that "Canada's fisheries are a 'common property resource', belonging to all the people of Canada" and that "it is the Minister's duty to manage, conserve and develop the fishery on behalf of

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Canadians in the public interest". Traditionally, non-governmental participation in the decision-making process was restricted largely to members of the fishing industry, but the new governance structure should, as it begins to be implemented, provide a greater role for members of the public and representative organizations in decision-making in recognition that the resource is public property and hence should be managed for long-term public good. Although this new direction is recognized and progressing in specific fisheries, there have been very few institutional changes to entrench this new governance structure in all future fisheries. Public influence on fisheries management may also come about through ecocertification of fisheries deemed to be sustainable and ecolabelling of products from these fisheries.

Recent Canadian fisheries management policy statements have embraced PA implementation, sustainable fisheries management, and increased public participation in the decision-making process. However, with few exceptions, Canada has not implemented effective harvest strategies to convert policy on sustainability into action. A sticking point to progress with regard to implementing sustainable fisheries management in Canada is reticence to commit to a predetermined harvest strategy (e.g., one based on a feedback harvest control rule) within an open and transparent decision-making forum. A predetermined harvest strategy is essential under the PA, is necessary for demonstrating sustainability, and is required to achieve ecocertification.

The term "sustainable", while ever present in policy statements, suffers greatly from ambiguous use. We provide a less ambiguous definition that can readily translate into explicit management actions. We give a brief review of national and international statutes, agreements and codes of conduct relevant to sustainable development, the precautionary approach, ecosystem approaches to fisheries, and ecocertification and distill the main guiding principles from these documents. We show that within the well-known and widely accepted theoretical basis for sustainable fisheries management, fishing can be considered sustainable over a broad range of use, from very little to the level where the resource is barely viable. However, we argue for a more circumscribed definition to be consistent with societal objectives expressed in national policy and international agreements. To move from policy to action, we outline a harvest strategy framework for sustainable fisheries management recently developed within Fisheries and Oceans Canada. This framework is consistent with Canadian policy and international agreements on sustainability and the PA, but needs to be fleshed out in detail on a stock-by-stock basis to become operational. The framework is designed to be flexible enough to accommodate differences across various fisheries and management systems. In our opinion, implementation of a framework such as this is long overdue in Canada, and we suggest a series of practical steps that could be taken to move forward.

International agreements

Canada has adopted a number of international agreements and undertakings, the body of which indicate the nation's philosophy towards fisheries management. There is a tendency towards increasingly conservation-minded approaches associated with sustainable development, the PA, and a broader ecosystem-based approach.

The Brundtland Report from the United Nations World Commission on Environment and Development (WCED) defines sustainable development as "development that meets the needs of the present without compromising the ability of future generations to meet their own needs" (Brundtland 1987). This definition recognizes that economic growth and development will result in changes to the ecosystem, but that it may not be bad provided that exploitation has been planned and the effects have been taken into account. It argues that renewable resources such as forests and fish stocks need not be depleted below levels at which maximum benefit is attained, provided that exploitation rates stay within limits determined by regeneration and natural growth. The Brundtland interpretation of sustainable development also takes into account the fact that renewable resources are part of a complex and interlinked ecosystem and that maximum sustainable yield (MSY) must be defined after taking into account the system-wide effects of exploitation.

Canada has ratified the legally binding United Nations Fish Stocks Agreement (UNFSA; United Nations 1995) that aims to ensure the long-term conservation and sustainable use of straddling and highly migratory fish stocks, the principles of which have subsequently been applied both nationally and internationally to fish stocks in other categories. Under Article 5, General Principles, it states that compliance with the Agreement requires that signatories adopt measures to ensure long-term sustainability and promote the objective of optimum utilization. Such measures are required to be based on the best scientific evidence available and should be designed to maintain at, or restore stocks to, levels capable of producing MSY, as qualified by relevant environmental and economic factors. UNFSA also requires signatories to apply the PA and to maintain or restore populations to above levels at which their reproduction may become seriously threatened. Annex II of the Agreement further states that the fishing mortality rate that generates maximum sustainable yield, F_{MSY}, is the minimum standard for limit reference points (i.e., fishing mortality should not exceed F_{MSY}). For stocks that are not overfished, fishery management strategies need to ensure that future fishing mortality does not exceed the F_{MSY} level and that the biomass does not fall below a predefined threshold. For overfished stocks, the biomass that would produce MSY, B_{MSY}, constitutes a rebuilding target.

Canada supports the Food and Agriculture Organization of the United Nations (FAO) Code of Conduct for Responsible Fisheries (FAO 1995*a*), a voluntary, nonbinding international agreement. The Code prescribes sustainable utilization of fisheries resources in harmony with the environment through application of the PA. FAO Guidelines for the PA (FAO 1995*b*) advocate a comprehensive management process that includes data collection, monitoring, research, enforcement, and review. Prior identification of desirable (target) and undesirable (limit) outcomes must be carried out, and measures are required that will avoid undesirable outcomes with high probability and correct them promptly should they occur. The Guidelines suggest that this be achieved through decision rules that specify in

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advance what action should be taken when specified deviations from operational targets are observed (i.e., harvest control rules). Furthermore, the Guidelines suggest that a management plan should not be accepted until it has been shown to perform effectively in terms of its ability to avoid undesirable outcomes (for example, through simulation trials). Lastly, the absence of adequate scientific information should not be used as a reason for postponing or failing to take measures to conserve target species and associated or dependent species, as well as nontarget species and their environment.

Canada was the first industrialized country to ratify the UN Convention on Biological Diversity (United Nations 1992). The Convention has three main goals: conservation of biodiversity, sustainable use of the components of biodiversity, and fair and equitable sharing of the benefits arising from this use. The 2002 World Summit on Sustainable Development in Johannesburg followed up on progress of the 1992 Convention (United Nations 2002). At the Johannesburg Summit, Canada agreed to attempt to achieve sustainable fisheries by maintaining stocks at, or restoring stocks to, levels that can produce MSY, with the aim of achieving these goals for depleted stocks on an urgent basis and where possible not later than 2015.

At the International Conference on the Governance of High Seas Fisheries and the United Nations Fish Agreement – Moving from Words to Action, hosted by Canada in St. John's, May 2005, the Minister of Fisheries and Oceans Canada, together with ministers of fisheries from a number of other countries, reaffirmed their commitment to implementation of the Johannesburg resolution to achieve sustainable fisheries by 2015 (Fisheries and Oceans Canada 2005*a*). They also resolved to implement a decision-making process that relies on the best scientific information available, incorporates the PA, and introduces ecosystem considerations into fisheries management.

Ecolabelling

The importance of marine capture fisheries needs to be emphasized. It is the last significant human food-gathering activity on a commercial scale from a naturally regenerating resource. There is an expectation that if carried out responsibly, marine fisheries will provide a long-term sustainable source of natural healthy food relatively free of growth hormones, antibiotics, pollutants, and the excesses of genetic selection. Ecocertification and ecolabeling are based on the assumption that the public is sufficiently concerned that it will moderate purchases on the basis of whether or not a seafood product meets a specified set of sustainability criteria. Philosophically, this has some similarities with the ideas behind fair-trade coffee and clothing.

It is a matter of ongoing debate whether or not these approaches will be effective. Multiple certification bodies could emerge with dissimilar standards, not all of which may be rigorously based on scientific data and analysis. There is also no guarantee that broad international criteria for certification will be consistent with national objectives and policies. This could weaken the value associated with ecolabels and diminish the potentially positive market feedback with regard to achieving sustainable fisheries. Traceability of products poses a problem in a global economy where fish may be caught in one location, transported across the globe to be processed in a second location, and then transported to a distant third location to be marketed and sold. There is also a concern that a premium on ecocertified products could negatively influence the market if a cheaper, noncertified alternative is readily available.

In an attempt to set an international standard for ecocertification, FAO issued "Guidelines for the ecolabelling of fish and fishery products from marine capture fisheries" (FAO 2005). The Guidelines emphasize the need for adequate data and a comprehensive evaluation to determine the current state and trends of stocks under consideration. Implementation of the PA is required and long-term conservation and sustainable use of fisheries resources is paramount. Stocks under consideration for ecolabelling must not be overfished and must be maintained at a level that promotes optimal utilization and continued availability for future generations. In the event that biomass drops below target levels, the Guidelines require that management allows for restoration to target levels within reasonable time frames. More broadly, ecolabelling exercises should also address adverse impacts of the fishery on the ecosystem.

Although the FAO Guidelines on ecolabelling emphasize the role of state and regional fisheries management organizations in the ecocertification process, the initial emphasis has come mainly from consumers, industry, and environmental nongovernmental organizations. Suppliers of fish products are being required by an increasing number of buyers to demonstrate that their products meet such standards. The most prominent certification process is the one developed by the Marine Stewardship Council (MSC), initially established in 1997 and operating independently since 1999. MSC has a scientific technical advisory board that advises a main board on setting and reviewing the MSC certification standard. MSC Principles and Criteria for Sustainable Fishing (Marine Stewardship Council 2002) are largely consistent with FAO Guidelines. They require that a fishery must be conducted in a manner that does not lead to overfishing or depletion, and for those populations that are depleted, fisheries must be conducted in a manner that demonstrably leads to their recovery. The intent is to ensure that productive capacities of resources are maintained at, or restored to, high levels designed to retain their long-term productivity and provide margins of safety for error and uncertainty. The MSC Principles and Criteria also require that fishing operations should allow for the maintenance of the structure, productivity, function, and diversity of the ecosystem (including habitat and associated dependent and ecologically related species) on which the fishery depends.

The MSC Principles and Criteria are largely consistent with recent Canadian fishery management policy statements described in the following section. This is mainly because both are guided by overarching principles defined in the UNFSA and the PA. Although the MSC certification process requires fisheries to be compliant with the PA, specific definitions of sustainable and depleted are not provided, leaving scope for proponents to ensure that fisheries seeking certification also comply with national policies. It is unclear how different conclusions regarding the degree of sustainability of a particular fishery arrived at by ecocertification and

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Fisheries and Oceans Canada assessment processes would be resolved. Clearly, openness, transparency, and peer review would be a consideration with regard to both processes. It is important to note that the ultimate responsibility for fisheries management in Canada remains with the Minister of Fisheries and Oceans Canada and is not being shifted to a third party such as the MSC.

National legislation and policies

There are three acts of parliament of relevance to the sustainable management of Canada's fisheries: the Fisheries Act, the Oceans Act, and the Species at Risk Act. The Fisheries Act (Government of Canada 1985) does not refer to the term sustainable but does require that activities respect the conservation and protection of fish, spawning grounds, and habitat. Canada's Oceans Act (Government of Canada 1996) requires sustainable development of the oceans and their resources and promotes the wide application of the PA as a framework for achieving sustainable development. The Species at Risk Act (Government of Canada 2002) fulfills a key national commitment under the UN Convention on Biological Diversity. It is aimed at preventing wildlife species from being extirpated or becoming extinct and provides for the recovery of wildlife species that are extirpated, endangered, or threatened as a result of human activity. It also requires that species of special concern be managed to prevent them from becoming endangered or threatened.

The Privy Council Office (PCO) of the Government of Canada has developed a Federal Framework for the PA to ensure that precaution is applied consistently across all disciplines in government (Government of Canada Privy Council Office 2003). This framework became government policy in 2003 with the intent that it be applied when a decision is required, there is a high associated uncertainty, and there is a risk of serious or irreversible harm. In such cases, decisions should err on the side of caution. For marine fish populations, reducing spawning biomass to the extent that the probability of poor recruitment has increased (recruitment overfishing) is considered a sufficient definition of serious harm (Fisheries and Oceans Canada 2002, 2004*a*). We suggest that this definition is too narrow because it focuses on the short-term risk of an undesirable outcome. In contrast, the defining UN and FAO PA documents paint a broader picture. They require that a comprehensive management plan be put in place that includes (*i*) targets of where one wants to be, (*ii*) limits that define where one does not want to be (i.e., serious harm), (*iii*) a pre-agreed harvest strategy to avoid limits and achieve targets, and (*iv*) a demonstration (for example, through simulation testing) that the strategy has a reasonable chance of success. Thus, the internationally accepted definition of the PA is more encompassing than the Privy Council Office interpretation.

Fisheries and Oceans Canada recently released two policy documents that describe general principles to be applied in managing fish populations and fisheries. The first is "A Policy Framework for the Management of Fisheries on Canada's Atlantic Coast" (Fisheries and Oceans Canada 2004*b*), and the second is "Canada's Policy for Conservation of Wild Pacific Salmon" (Fisheries and Oceans Canada 2005*b*). Both documents reiterate that the Minister of Fisheries and Oceans Canada, on behalf of all Canadians, has complete discretion with regard to resource conservation and sustainable use of fisheries resources and their habitat and for the access and allocation thereof. However, both were developed through extensive consultations with provincial and territorial governments, First Nations, the fishing industry, and others, i.e., they reflect a more open governance structure. They encourage further development of specific management strategies tailored to the needs and aspirations of those involved in using the resource.

The Atlantic fisheries policy document focuses on commercial fisheries for Atlantic groundfish species, the dominant fishery being that for Atlantic cod. It incorporates the general principles of the PA as defined in UN and FAO documents. Highest priority is given to conservation and rebuilding of marine resources. Conservation is defined as "sustainable use that safeguards ecological processes and genetic diversity for present and future generations". The Atlantic fisheries policy requires stakeholders to participate directly in management of the fishery and share in accountability for outcomes through a shared-stewardship process. The operational harvest strategy within the framework will include limit and target reference points and rules for scaling resource use to stock conditions to avoid undesirable outcomes and to rebuild depleted stocks.

The Pacific salmon policy focuses on the conservation of wild populations. Unlike the Atlantic fisheries policy, the terms conservation and sustainable are defined separately. Conservation is defined as "the protection, maintenance, and rehabilitation of genetic diversity, species, and ecosystems to sustain biodiversity and the continuance of evolutionary and natural production processes". Sustainable use is defined as "the use of biological resources in a way and at a rate that does not lead to their long term decline, thereby maintaining the potential for future generations to meet their needs and aspirations". Conservation, therefore, takes on a broader meaning in the Pacific salmon policy than it does in the Atlantic fisheries policy. This is an evolutionary step recognizing obligations under Canada's Species at Risk Act and the UN Convention on Biological Diversity. The Pacific salmon policy considers three status zones, designated red, amber, and green, separated by status benchmarks. The lower benchmark, between the red and amber zones, is at a status designation high enough to ensure that there is a substantial buffer between it and any level of abundance that could lead to a conservation unit being considered at risk of extinction by COSEWIC (Committee on the Status of Endangered Wildlife in Canada), the independent advisory body under the Species at Risk Act. The higher benchmark, between the amber and green zones, identifies whether harvests are greater or less than the level expected to provide maximum annual catch from the conservation unit. The red zone is undesirable because there is a risk of extirpation, whereas the amber zone implies that caution in management should be exercised. The Pacific salmon policy is not prescriptive regarding management actions or harvest control rules in each status zone, recognizing that economic and social needs are to be balanced against conservation objectives when these actions and rules are formulated. Consultation is ongoing to make the policy operational.

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Guiding principles for sustainable fisheries management

National policy and international agreements reviewed above can be distilled into a number of important guiding principles that can form the basis for developing and implementing operationally explicit sustainable management strategies for wild marine capture fisheries.

(*a*) Fisheries management should make use of the best available scientific information and should aim to achieve both conservation and sustainable-use objectives.

(*b*) Conservation should give high priority to the protection, maintenance, and rehabilitation of genetic diversity within both species and ecosystems. This must extend to all species, not only those of immediate commercial importance, but also those caught incidentally or negatively impacted by fishing operations.

(*c*) Sustainable use should be based on predetermined management plans that respond to changes in stock status. This can best be achieved by implementing a harvest strategy framework, incorporating biological reference points (limits and targets) and feedback harvest control rules. Control rules should maintain the stock in the vicinity of the target with high probability, while allowing only very low probability of falling below the limit reference point at any time. The harvest strategy should ensure that stocks below target reference points rebuild within prescribed time frames.

(*d*) Harvest control rules need to be evaluated through simulation testing, preferably prior to implementation, to determine whether they are likely to perform satisfactorily under a variety of fishery behaviours, change in stock size and productivity, and range of uncertainty.

(*e*) Conservation and sustainable use involve trade-offs. In keeping with changing governance policy towards openness and transparency, management strategies for wild marine capture fisheries should be developed in cooperation with First Nations, stakeholders, and groups representative of public interests (e.g., conservation, recreation, etc.) to fully reflect societal values.

Recent developments in Canada with respect to the Atlantic fisheries policy and the Pacific wild salmon policy demonstrate intent to embrace these principles. More detailed implementation plans are now required to move from policy to action.

From policy to action

Policy documents are peppered with the term sustainable; however, there is very little detail on what the term actually means and how sustainability should be achieved in practice. A widely applied theoretical basis for sustainable fisheries management is the Graham (1935) – Schaefer (1954) model in which the equilibrium surplus production (yield) is a concave and symmetrical function of fishing effort and stock biomass. In practice, data for most commercially exploited stocks suggest that the surplus production function is asymmetrical. Regardless of the actual shape of the function, fishing is sustainable under this model over a broad range of use. At the lower end of the range, a very low catch may be sustained with very little fishing mortality. At the opposite extreme, a similar catch may be sustained, but at a much higher fishing mortality such that the population is maintained at a low biomass near the brink of extinction. Clearly, this range encompasses foregone opportunities at one end and wasteful practices with a high risk of disaster at the other. The level of use that would provide the desired benefits to society without compromising the conservation of the stock lies somewhere within this range and needs to be identified to sharpen our definition of sustainability and to direct fisheries management actions.

The peak of the surplus production function is termed maximum sustainable yield (MSY). The associated fishing mortality and equilibrium biomass at which this occurs are F_{MSY} and B_{MSY}, respectively. Economic considerations related to the costs and revenues from harvesting the resource compared with the value placed on conserving fish stocks and maintaining ecosystem functioning may result in a perceived societal optimum that differs from MSY. Under simple economic assumptions, a stock above B_{MSY} requires less fishing effort to obtain an equivalent harvest relative to a stock below B_{MSY}, giving rise to lower harvesting costs and, consequently, higher economic rent. Maximum economic rent is achieved at the point at which marginal cost equals marginal revenue and this occurs at a stock size above B_{MSY} and a fishing mortality below F_{MSY}.

Consideration of an economic discount rate can have a major impact on fisheries management decision-making (Clark 1985). If a normal business discount rate of, for example, 10% is assumed, then the optimal stock size may be below B_{MSY} and the optimal harvest rate may be above F_{MSY}. For an unproductive resource in which the intrinsic rate of increase is less than the discount rate, there is incentive to fish the stock to extremely low levels. However, Clark (1985, p. 27) points out that the social rate of discount is usually less than the business rate, leading some authors to argue that "consideration of intergenerational equity requires the use of zero discount rate in public conservation decisions". Discount rate is not explicitly considered in the UNFSA, the Johannesburg Accord, and the two recent Canadian fishery policy documents. Canadian ratification of the UNFSA and its default management targets of B_{MSY} and F_{MSY} indicate support for the use of a social discount rate close to zero.

Other economic considerations may also have important impacts on the fishery, which may not be consistent with sustainability (Clark 1973). In a competitive open-access fishery or a fishery with ineffective entry or capacity controls, fishing effort will tend to increase until economic rent is dissipated or alternative employment becomes more attractive. Depending on the lag in response by the stock to fishing, existence of alternative employment, current value of the harvest, and the cost of fishing, a stock may be depleted well below B_{MSY}. Overcapacity in a competitive fishery can be a major impediment to achieving sustainability. Subsidies and income-support programs designed to meet other societal objectives such as enhancing coastal communities and protecting sovereignty may lower the cost of fishing, allowing effort and capacity to be maintained on a declining stock and resulting in a propensity for greater depletion. Note that even in a rights-based fishery in which

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there is some form of tenure and competition is largely eliminated, stocks may be depleted relative to B_{MSY} depending on the perceived discount rate, as discussed above. The propensity for stock depletion may increase in mixed-species fisheries and in fisheries where bycatch of other valuable species occurs.

Societal value placed on maintaining biodiversity and ecosystem functioning is an increasingly important consideration. In contrast to the fishing industry, society in general is likely to place more emphasis on recreational value, non-use value, and safeguarding future options, which tends to drive the perceived optimum stock size above B_{MSY}. In terms of ecosystem functioning, theoretical studies suggest that MSY from a system of interacting species will be less than the sum of individual MSY values computed on the basis of single-species models (May et al. 1979; Beddington and Cooke 1982). Given that one cannot maximize the sustainable yield from all species simultaneously, fisheries management under an ecosystem-based approach should be more conservative than would be predicted based on single-species assessment models by keeping stocks above B_{MSY}. In many cases, current fishing mortality exceeds that required to achieve MSY on a species-by-species basis. This led Mace (2001) to suggest that reducing fishing mortality to below F_{MSY} would be a positive first step in moving towards ecosystem approaches to fisheries (EAF).

Management plans need to be revised to include a predetermined, operationally explicit, harvest strategy to move from policy to action with regard to sustainable fisheries. We describe a general harvesting strategy framework that has recently been developed by Fisheries and Oceans Canada and suggest how it may be made operationally explicit.

Harvest strategy

Canada has developed a flexible harvest strategy framework for implementing the minimal requirements of the PA for wild capture fisheries to meet international standards (Fisheries and Oceans Canada 2006; Fig. 1). The upper stock reference (USR) is the stock size (in units of spawning stock biomass or equivalent) below which the removal rate is reduced to promote recovery and thus ensure a low risk of serious harm. Stocks above the USR are considered to be in a healthy zone. The limit reference point (LRP) is the stock status level below which productivity may be sufficiently impaired to cause serious harm but above the level at which the risk of population extinction becomes a concern. The stock status zone below the LRP is called the critical zone. The stock status zone above the LRP but below the USR called the cautious zone.

The removal reference (RR) is the maximum acceptable removal rate in terms of the ratio of all human-induced removals to total exploitable stock size (in units of fishing mortality rate or equivalent). Under UNFSA, the fishing mortality rate that generates MSY (F_{MSY} or equivalent) is a minimum standard for a PA fishing mortality limit reference point. Therefore, the RR under the Canadian Framework should be less than F_{MSY} irrespective of stock status. Fishing mortalities set at or below F_{MSY} will result in the spawning stock biomass fluctuating around B_{MSY} or above. In the cautious zone, fisheries management actions should promote

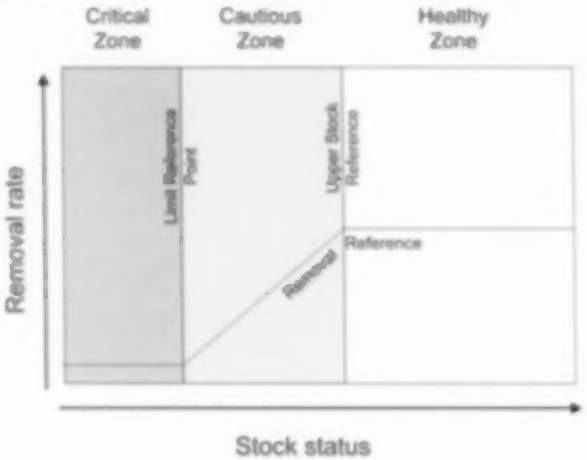

Fig. 1. A general framework for harvest strategy compliance with the precautionary approach developed by Fisheries and Oceans Canada. Vertical lines represent reference points demarcating healthy, cautious, and critical zones. The horizontal line in the healthy zone demarcates a removal rate limit that should, under the United Nations Fish Stocks Agreement, correspond to F_{MSY} or its proxy or less. Removal rate decreases through the cautious zone with decreasing stock size and is as close to zero as possible in the critical zone (broken line).

stock rebuilding to the management target located in the healthy zone (i.e., to the vicinity of B_{MSY} or above). To achieve this, the RR should progressively decrease as the stock level approaches the LRP following some prescribed harvest control rule that takes into account the risk of falling into the critical zone and the probability of recovery to the healthy zone. In the critical zone, the status of the stock has declined to such a low level that serious harm may have been done and the stock is thus in a precarious state. In this zone, conservation is a first priority and fishery management must promote stock growth by keeping human removals at the lowest level possible.

Technically, a fishery could be considered to be sustainably managed in any one of the three stock status zones. However, we argue that only fisheries in the healthy zone being managed at a harvest rate below F_{MSY}, or fisheries in the cautious zone being managed at a harvest rate demonstrated to be low enough to rebuild the stock to the healthy zone with a high probability in an acceptably short period of time, can be considered to be sustainably managed in accordance with Canadian fisheries policy and international agreements.

To make the harvest strategy described above fully operational, it has to be fleshed out in more detail on a stock-by-stock or fishery-by-fishery basis. It then needs to be implemented and the performance monitored. Closed-loop simulation testing (e.g., Martell and Walters 2002) prior to implementation is strongly advised to determine the potential robustness of the strategy to various forms of uncertainty such as observation error, estimation error, model misspecification, and natural variability. This form of testing has been advocated by the International Whaling Commission since the late 1980s (Butterworth 2007) and is gaining

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broader international recognition as management strategy evaluation (MSE; Kell et al. 2007). A management strategy is the combination of stock status data, a method to analyse these data, a decision rule to convert the analysis results into action, and implementation. Candidate strategies are evaluated for robustness to uncertainties about the underlying fisheries system and for performance relative to fishery objectives.

Some progress has been made on a few Canadian stocks with regard to determining LRPs based on an interpretation of serious biological harm that may be difficult to reverse. However, USRs and harvest control rules require broader consideration of management objectives, risk tolerances, and performance measures, and almost no progress has been made. We suggest operationally explicit defaults for a stock for which there is an analytical (quantitative, model-based) assessment. We draw on the proposed harvest strategy standard for New Zealand fisheries (New Zealand Ministry of Fisheries 2007), recognizing that a comparable initiative to develop such a standard has yet to commence in Canada. For stocks for which there are insufficient data to develop analytical assessments and apply the suggested defaults, suitable proxies need be developed to attempt to capture the intent of the defaults.

The proposed New Zealand (NZ) standard has three core components. The first is a specified target around which the stock should fluctuate. The default target is B_{MSY} or better ("or better" means more conservative in all cases). The second component is a soft limit that triggers a requirement for a rebuilding plan that would recover the stock to the target within a specified period of time. The default soft limit is 50% B_{MSY} or 20% B_0, whichever is greater. The third component is a hard limit below which fisheries should be considered for closure. The proposed default is 25% B_{MSY} or 10% B_0, whichever is greater. The standard advocates using MSE to ensure that candidate management strategies have (*i*) the probability of achieving the target of at least 50%, (*ii*) the probability of breaching the soft limit that does not exceed 10%, and (*iii*) the probability of breaching the hard limit that does not exceed 2%. The NZ standard suggests that (*ii*) and (*iii*) could be simplified within the MSE to a probability of no more than 5% of breaching the soft limit.

There are clear similarities between the New Zealand standard and the Canadian framework. The NZ standard target stock size of B_{MSY} or better is a reasonable default target for Canada. There is not an equivalent soft limit in the Canadian framework to demarcate the point at which a "formal, time-constrained rebuilding plan" is triggered. However, there is a requirement for a recovery plan under the Species at Risk Act for Canadian stocks that are deemed to be at risk of extinction, and we see this as an important control mechanism to initiate management actions to reverse stock declines before reaching a situation where serious harm to the stock may occur. The NZ standard hard limit is similar to the Canadian LRP, and the proposed default values are acceptable. Combining the soft and hard limits given, as suggested in the NZ standard, and using a 5% probability as the evaluation criterion in MSE shifts emphasis to the higher soft limit.

The Canadian framework requires an explicit relationship between stock size and removal rate in the cautious zone, whereas this is not prescribed in the NZ standard. In both the NZ standard and the Canadian framework, it is clear that to have at least a 50% probability of fluctuating around a target of B_{MSY} or better, the highest acceptable fishing mortality would have to be F_{MSY} or less. Furthermore, F_{MSY} is considered a minimum standard for a limit reference point under UNFSA. It is also clear that if the stock size falls below the target because of unfavourable environmental conditions affecting recruitment or growth, the removal rate will have to be set at a level that will promote rebuilding. In both the NZ standard and the Canadian framework, candidate management strategies will therefore include decision rules that vary the removal rate in relation to stock status.

There are at least two ways in which the notion of risk and risk management can be introduced in the implementation of the harvest strategy framework. The first is to determine the state of the stock and then provide risk plots for short-term projected stock sizes relative to reference points under a range of total allowable catch (TAC) options as a guide to decision-making (Walters and Punt 1994; Gavaris and Sinclair 1998). Typically, the risk of biomass falling below the USR or fishing mortality falling above the predefined RR would be evaluated in the healthy zone. The risk of biomass falling below the LRP or fishing mortality falling above the RR would be considered in the cautious zone. Our experience in providing this kind of scientific advice on numerous Canadian groundfish stocks over the last 10 years has shown that fisheries managers have difficulty interpreting risk plots. Consequently, TAC decisions are often irrationally risk prone on a declining or depleted stock. Critics of this approach stress that estimates of current stock size may be very uncertain and the appropriateness of selected reference points may be questionable (Hilborn 2002) and that projections commonly ignore structural or model uncertainty, resulting in risk advice that may be of limited value (Butterworth 2008).

An alternative approach is to use MSE as a comprehensive risk management tool within an open and transparent decision-making environment. The MSE simulations would include conservation and sustainable use performance measures. Only those strategies that meet the conservation standards described above would be considered for implementation. Sustainable use performance measures would include measures of average catch and interannual variability in catch. Uncertainties regarding the true state of nature would be limited to plausible alternatives that reflect the best available knowledge. Evaluation of alternative strategies would be done collectively by stakeholders to ensure acceptance and representation. It is inevitable that there will be debate on the trade-off between conservation and sustainable use performance measures during the evaluation process. Rather than developing an overall weighted mean performance measure to determine a "winner", it may be more useful to eliminate those management strategies that fall outside an acceptable range for any one performance measure and then discuss the relative merits of those that satisfy the acceptability conditions. Experience with this approach indicates that those strategies that often perform best tend to involve a relatively simple interpretation of annual data to make decisions, but that there is a need for periodic re-evaluation of the strategy as new knowledge and

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experience are gained and incorporated into the operating model (Butterworth and Punt 1999).

Implementation

Although much good policy on sustainable fisheries management exists, both within the international community and nationally, implementation on marine capture fisheries off the coasts of Canada has occurred slowly, if at all. Limit reference points have been defined for only a few stocks. Other aspects of the PA framework, including the harvesting strategy, remain to be fleshed out in nearly all cases. Consequently, the majority of existing fisheries management plans in Canada cannot be demonstrated to be consistent with international sustainability or PA standards. A number of fisheries are being pursued in the absence of any scientific assessment of sustainability because of lack of data and resources. This needs to be rectified. In other cases, some data exist but are insufficient to obtain quantitative estimates of absolute stock size or MSY-related reference points. In these cases, relative indices and proxy reference points should be applied to implement basic precautionary harvest strategies, while striving to collect more and better data to provide more reliable quantitative estimates of stock status, reference points, and risk in the future. In yet other cases, there are sufficient data to carry out a comprehensive analysis and to develop harvest strategies that can be demonstrated to be robust to uncertainty and to meet PA and sustainability requirements while still allowing commercially viable fisheries. These cases teeter on the brink of implementation, held back by a reluctance to discard current largely ad hoc nonprescriptive approaches despite ample demonstration that these approaches are ineffective.

Canada is committed to implementing sustainable fisheries management in accordance with a growing list of international and national statutes and accords. The Bruntland definition of sustainable development has been adopted in two recent Fisheries and Oceans Canada policy statements covering Atlantic groundfish and wild Pacific salmon. Canada has ratified the UNFSA and the UN Convention on Biological Diversity and endorsed the PA to fisheries management as outlined by FAO, as well as signed the Johannesburg accord regarding managing fisheries at MSY. Recent new national statutes have further emphasized this commitment by recognizing the PA in the Oceans Act and the protection of species from extinction in the Species at Risk Act. The Privy Council Office of Canada reviewed the application of the PA across all government activities and the application to capture fisheries has been accepted. A general harvest strategy compliant with the PA has been developed. Fisheries and Oceans Canada is also committed to using an open, transparent, and inclusive governance process for implementing fisheries management strategies in accordance with these accepted agreements.

There are a number of important guiding principles for implementing sustainable management that emerge from these formative documents: (*i*) management should be based on the best available science; (*ii*) conservation should receive the highest priority; (*iii*) sustainable use should be based on predetermined plans that respond to changes in stock status; (*iv*) management strategies should be evaluated through simulation testing; and (*v*) conservation and sustainable use trade-offs should be reconciled in an open, transparent, and inclusive manner.

Despite this strong commitment, progress toward effective implementation has been slow compared with how long these concepts have been around. We have noted through several years of experience that some important changes in governance have been made. Scientific meetings that used to occur in camera are now open to interested stakeholders. The top-down process that dominated decision-making in the 1970s and 1980s is changing, but change is slow. This more open process has resulted in increased communication among stakeholders, managers, and scientists, but being open and transparent means listening to other opinions and objectives, finding common ground, and seeking resolution to conflicts. This has been time consuming; the required institutional changes have not yet occurred, and more work is required.

Sharpening the definition of sustainable fisheries management will hasten the implementation process. Under the current vague definition, fisheries may be considered sustainable over a very broad range of use spanning foregone benefits at the low end and commercial extinction, or worse, at the other end. We can do much better. We endorse the New Zealand MSY-based harvest strategy standard as a good starting point for an operationally explicit Canadian framework. Although the Canadian Fisheries Act does not mention MSY, the body of international and national statutes and agreements that Canada has ratified and accepted is sufficient to require accountability with regard to this objective. To harvest populations at a rate that exceeds F_{MSY} and to maintain fish stocks below B_{MSY} for extended periods of time with no commitment to rebuilding makes no sense at the fish population, ecosystem, economic, or societal level. To move forward on the implementation of sustainable fisheries management in Canada, a sharper definition of sustainability is required to ensure that fisheries management clearly "meets the needs of the present without compromising the ability of future generations to meet their own needs."

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